Soybean Genetics Newsletter

Volume 22 May 1995

USDA-Agricultural Research Service
Department of Agronomy
and Department of Zoology / Genetics
Iowa State University
Ames, Iowa 50011

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UNITED STATES OF AMERICA

Foreword

Volume 22, 1995 of the Soybean Genetics Newsletter has been made possible by the diligent efforts of Terry D. Couch, USDA Agricultural Research Technician. He deserves our sincere appreciation for his efforts.

-Reid G. Palmer, editor
USDA-ARS-FCR

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NEW BOOK

"COMPENDIA OF CHINESE SOYBEAN VARIETIES"
VOLUME II (1978-1992)

Following the first volume of "Compendia of Chinese Soybean Varieties" published in 1985, the second volume is now available. It mainly covers the soybean varieties released during 1978-1992 in China. 738 varieties are included with detailed descriptions of botanical and agronomic characters. 197 photos also are attached. The book is edited by the Soybean Institute, Jilin Academy of Agricultural Sciences and published by Agricultural Publishing House in China. It has 421 pages written in Chinese. The price is $35.00 U.S. dollars including shipment costs. All subscriptions and payments should be mailed to:

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1995 Soybean Germplasm Crop Advisory Committee Report

The Soybean Germplasm Crop Advisory Committee (SGCAC) held its annual meeting February 20, 1995, at the Soybean Breeders' Workshop at St. Louis, MO. In attendance were committee members: Thomas Devine, William Kenworthy, Randall Nelson, Lawrence Young, Thomas Kilen, E. E. Hartwig, Michael Kenty, Tom Welacky (representing Gary Ablett), H. Roger Boerma, John Thorne, Bob Keeling (representing John Hicks), Fritz Schmitthenner, Dan Phillips, and John All. In addition, guests Mark Bohning, James Orf, Scott Abney, James Specht, and Perry Cregan were present. Chairperson, Tom Devine, called the meeting to order at 8:00 am.

The committee broke into subcommittees until 9:30 a.m.

Subcommittee reports:

Operations (All, Chair, Bohning; Hartwig; Welacky).

Bohning reported that The GRIN system has recently been placed on INTERNET AND WEBB, which has broadened the user community. The PC version of GRIN is available free. Ways to improve GRIN and INTERNET by making them more user friendly, easier to access, and "interconnectable" were discussed. Broadening the user community might be accomplished by expanding the information in GRIN by including data from state trials or other information of interest to extension personnel, seed marketers, farmers, and others. The GRIN Newsletter distribution might also be expanded by sending to The Soybean Genetics Newsletter, University Libraries, Extension locations, and The Soybean Digest, as well as putting it on INTERNET.

The printing and distribution of a table of recent additions to the Soybean Collection was discussed. Possible distribution beyond traditional recipients might include INTERNET, GRIN, The Soybean Genetics Newsletter, and an ASA note.

The subcommittee discussed the Primary User Community for information in GRIN. Soybean breeders (university, federal, and private), molecular geneticists, cooperative extension personnel, seed marketers, consultants, and farmers might all be included.

Acquisition (Boerma, Chair, Nelson; Kenworthy; guests, Cregan, Orf, Specht).

Boerma moved that the SGCAC approve a resolution supporting a trip by Theodore Hymowitz, Univ. of Illinois, to central Australia to collect perennial Glycine species. The motion passed.

The issue of putting maplines (lines from populations used in genetic studies involving molecular markers) in the germplasm collection was discussed. The subcommittee recommended that maplines be eligible to enter the collection under the following procedures:

1. A request would be sent to the SGCAC Chair that a series of maplines be considered. The originator must be willing to freely share molecular data for the request to be considered.

2. The SGCAC would make a preliminary determination whether the lines should be considered for eventual inclusion.

3. If the SGCAC determines that there is potential value for the lines, the originator would maintain the lines for 5 years, make them available to researchers on request, and keep distribution records.

4. After 5 years, the SGCAC would make a final determination whether to include the lines in the collection.

This proposal was approved by the SGCAC.

Evaluation (Kilen, Chair; Devine; Phillips; Schmitthenner; Thorne; Young)

The future of the Phytophthora sojae race collection currently maintained by Schmitthenner was discussed. The subcommittee drafted the following resolution which was approved by the SGCAC.

"Recognizing that phytophthora root rot disease currently results in very costly losses to the U.S. soybean crop and continually poses the threat of major devastation to U.S. soybean production averted only by the use of genetically resistant cultivars, and

Recognizing that the breeding of such cultivars is dependent upon the ability to distinguish the many particular genes for resistance to the several specific pathogenic races of the fungal pathogen, and

Recognizing the necessity of maintaining and monitoring working collections of specific isolates of the several pathogenic races in order that soybean breeders may have available the isolates needed to develop new resistant cultivars to protect against the ever changing new races of the pathogen, and

Recognizing the recent loss of important collections with the retirement of experiences plant pathologists and the imminent danger of further losses to the critical element in the infrastructure of U.S. soybean production posed by the anticipated retirement of additional pathologists,

This committee strongly recommends that complete collections of cultures of Phytophthora sojae be maintained at 3 locations in areas where the disease is prevalent. We recommend that working collections be maintained by plant pathologists actively involved in research with the disease at 2 locations in northern states and 1 in a southern state with adequate funding to insure long term continuance of the projects."

This resolution should be sent to the National Program Leaders Oilseeds and Bioscience and Plant Health, and to other appropriate concerned individuals and groups.

Schmitthenner presented a proposal for a trip to China to search for Phytophthora sojae in Chinese provinces where soybeans are a major crop or where Phytophthora resistance is common. The SGCAC strongly recommended approval of this trip.

The continuing need for development of a core collection of soybean germplasm was discussed.

The continuing need for screening techniques for resistance to southern green stinkbug and silverleaf whitefly was discussed.

The subcommittee recommended that a site be chosen for annual evaluation of part of the collection for resistance to aerial webb blight.

The increased importance of sclerotinia stem rot (white mold) was discussed. The committee recommended support for the refinement of evaluation techniques and implementation of a program for screening germplasm, with initial emphasis on the early maturities, for resistance.

Phillips will form a subcommittee to prioritize research needs and to seek funding for significant disease problems of soybeans.

Chinese Germplasm Exchange

Nelson reported that about 500 new G. max accessions from southern China were grown for the first time in 1994 at Stoneville.

USDA Soybean Germplasm Collection Report

Approximately 15700 accessions are now in the collection. In 1994, 7803 seed lots were distributed from the USDA Soybean Germplasm Collection in response to 266 requests. Evaluations of 340 Group I and earlier lines at Roseville, MN; 1031 Group I to IV lines at Urbana, IL; and 854 Group V and later at Stoneville, MS, were conducted in 1994. Thirty-four new accessions of G. soja were added to the collection in the Fall of 1994.

Election of Officers

Devine was nominated as SGCAC Chair for the 1995-96 and unanimously elected. Thorne was nominated as Vice-Chair for 1995-96 and unanimously elected.

Meeting adjourned 11:45

T. E. Devine, Chair
John Thorne, Vice-chair

Soybean Germplasm Crop Advisory Committee Members 1995-1996

Member	Area of Representation	Term Expires
Ablett, Gary Ridgetown College of Ag. Technology Ridgetown, Ontario NOP2C0 Ph.: 519-674-5456 FAX: 519-674-3504	Canadian Representative	1997
All, John University of Georgia Dept. Entomology Athens, Georgia 30602-7503 Ph: 706-542-7585 FAX: 706-542-2279	Entomology	1997
Ashley, Doyle University of Georgia Dept. of Crops Soil Sci. 3111 Plant Sciences Bldg. Athens, GA 30602 Ph: 706-542-0922 FAX: 706-542-0914	Physiology	1998
Boerma, H. Roger University of Georgia Dept. of Crop and Soil Sciences Athens, GA 30602-7272 Ph: 706-542-0927 FAX: 706-542-0914	Public Breeding, South	1996
Devine, Thomas E., Chair USDA, ARS, PSI., PMBL Bldg. 006, Rm. 118, BARC-W 10300 Baltimore Ave. Beltsville, Maryland 20705-2350	Cytogenetics and Molecular Genetics	1997
Hartwig, Dr. Edgar Soybean Production Research USDA, ARS P.O. Box 196 Stoneville, Mississippi 38776 Ph: 601-686-3126 FAX: 601-686-3140	USDA Germplasm Collection	Ex off

Hicks, John D Pioneer Hi-Bred Intern's P.O. Box 4428 Seven Oaks Road Greenville, Mississippi 38704 Ph: 601-335-9152 FAX- 601-335-9164	Private Breeding, South	1998
Kenty, Michael M. Soybean Production Research USDA, ARS P.O. Box 196 Stoneville, Mississippi 38776 PH: 601-686-9311 FAX: 601-686-5465	USDA Germplasm Collection	Ex off
Kenworthy, William J. University of Maryland Dept. of Agronomy College Park, Maryland 20742 Ph: 301-405-1324 FAX: 301-314-9041	Public Breeding, North	1997
Kilen, Thomas C. Soybean Production Research USDA, ARS P.O. Box 196 Stoneville, Mississippi 38776 Ph: 601-686-3125 FAX: 601-686-3140	USDA Germplasm Collection	Ex off
Nelson, Randall University of Illinois Dept. Agronomy, NSRL, USDA 1101 West Peabody Urbana, Illinois 61821 Ph: 217-244-4346 FAX: 217-333-4639	USDA Germplasm Collection	Ex off
Ogren, William Bldg 005 BARC West Nat. Prog. Staff USDA, ARS Beltsville, MD 20705 Ph: 301-504-5930 FAX: 301-504-5467	National Program Leader	Ex off

Phillips, Daniel Plant Pathology University of Georgia 1109 Experiment Station Griffin, GA 30223 Ph: 404-412-4009 FAX: 404-228-7203	Plant Pathology	1998
Thorne, John Northrup King Company P.O. Box 949 Washington, Iowa 52353-0949 Ph: 319-653-6645 FAX: 319-653-4609	Private Breeding, North	1996
Young, Lawrence D. USDA, ARS West Tennessee 38301 Ph: 901-425-4741 FAX: 901-425-4760	Nematology/Pathology	1996

1994 Soybean Germplasm Crop Advisory Committee Report

The Soybean Germplasm Crop Advisory Committee (SGCAC) held its annual meeting February 16, 1994, at the Soybean Breeder's Workshop in Memphis, TN. Members present: Mark Bohning, H. Roger Boerma, Bill Kenworthy, Dennis Egli, Jim Harper, John All, John Hicks, Lawrence Young, Fritz Schmitthenner, Gary Ablett, Claudia Coble, Michael Kenty, John Thorne and Tom Devine. chairperson, Tom Devine, called the meeting to order at 8:00 a.m. New committee members, John All and Gary Ablett were introduced. The re-election of Tom Devine and Bill Kenworthy to 3-year terms was announced. (Current committee members and term expiration dates are listed in the accompanying table).

The committee broke into subcommittees until 9:00 a.m.

Subcommittee reports:

Acquisition (Thorne, Chair; All; Young; Coble).

Coble gave an update on the addition of isolines to the germplasm collection. Dr. Hartwig added approximately 70 lines, some of which are isolines.

The private variety collection was discussed. Approximately 20 varieties are currently in the collection; no new entries have been added since 1990. Thorne will try and determine the interest in continuing this collection at the commercial Soybean Breeders (CSB) meeting on February 18. If there is sufficient interest, Randy Nelson will send a request for entries and a list of current varieties to the CSB membership. (Note: The general consensus of CSB was that the collection should be continued, and that most companies would be interested in contribution varieties).

The issue of adding lines which have been used in molecular mapping studies was discussed. Concerns included the following:

1. The potential for large numbers of lines.
2. Willingness of researchers to share this kind of material.
3. Criteria for selecting material for the collection.
4. Potential for loss of valuable material if a program is terminated.

Devine appointed a subcommittee chaired by Boerma with Coble, Devine and others as deemed appropriate by the chair, to discuss the issues related to collection of map lines, and to make recommendations to the committee.

Evaluation (Kenworthy, Chair; Devine; Kenty; Schmitthenner).

The importance of evaluation of the collection for Aerial Web Blight, incited by Rhizoctonia solani, a serious disease in parts of Louisiana which may be spreading into Mississippi and Arkansas, was discussed. Reliable screening methods for this disease have not been developed. The committee approved a resolution that the SGCAC encourage exploration of characterization of appropriate germplasm.

The need for screening material for resistance to sweetpotato whitefly was also discussed. The committee approved a resolution that the SGCAC encourage

evaluation for resistance to sweetpotato whitefly and the stinkbug complex and the characterization of appropriate germplasm.

The status of the development of a core collection was discussed. Major factors for stratification and compartmentalization of the collection for subsampling to construct the core collection will be the place of origin and maturity. These factors would constitute significant reproductive barriers to gene flow. the first task addressed was correct errors in the information bank for place of origin. At this point, only Asian accessions will be used for the core. Work will initially focus on the earlier maturity groups. Using location of origin and maturity as matrix coordinates 350 categories will be established for subsampling. The consensus of the SGCAC is that we encourage the continued development of a core collection.

Operations: (Boerma, chair; Ablett; Bohning; Egli; Hicks).

Bohning gave an update on changes in GRIN. The GRIN system expects to have a new computer and software operational by the end of June, 1994. Improvements include potential for linkage with other databases as well as speed and efficiency of the system. Bohning also reported that a PC version of GRIN is new available.

Germplasm Exchange with China:

The 500 lines from nine central provinces of China received in 1992 were evaluated for phytophthora root rot and are currently being evaluated for soybean cyst nematode resistance and molecular markers. Continued cooperation in exchange of germplasm and evaluation of germplasm are anticipated.

Devine appointed Kenworthy to draft letters of appreciation to the organizations contribution to the Chinese Germplasm Exchange Program.

USDA Soybean Germplasm Collection Report:

Approximately 13,500 Glycine max strains are now in the collection. In 1993, a total of 13,314 seedlots were distributed from the USDA Soybean Germplasm Collection in response to 342 requests from 37 states and 22 foreign countries. There were 295 domestic seed orders for 12,540 seedlots and 47 foreign requests for 889 seedlots.

Details of reports presented on the Chinese Germplasm exchange, the germplasm collection and germplasm evaluation at Jackson, Tennessee, and Stoneville, Mississippi, are presented elsewhere in the SGN.

Election of Officers: Devine was nominated as Chair of SGCAC for 1994-95 and unanimously elected. Thorne was nominated as Vice-Chair for 1994-95 and unanimously elected.

The meeting was adjourned approximately 11:30 a.m.

John Thorne, Vice-Chair
T. E. Devine, Chair

SOYBEAN GENETICS COMMITTEE REPORT

Minutes of Meeting Held February 20, 1995

The Soybean Genetics Committee (SGC) met from 8:00 to 9:20 p. m. at the Sheraton Plaza Hotel, St. Lous, Missouri, in conjunction with the National Soybean Breeders' Workshop.

Committee members attending the meeting were: P. B. Cregan, B. Diers, R. L. Nelson, C D. Nickell, J. H. Orf, T. W. Pfeiffer, and J. E. Specht. B. R. Hedges and T. W. Pfeiffer had been elected by mail ballot to serve a three-year term on the Committee. At the conclusion of the meeting, T. W. Pfeiffer was elected Chair for the year ending in February, 1996.

Also in attendance at the meeting was Dr. Ted Hymowitz. Current Committee members and February expiration dates for their terms on the Committee are:

Dr. B. Diers (1997)
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Dr. J. H. Orf (1997)
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Dr. T. W. Pfeiffer (1998) (Chair)
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Procedure

As in the past, manuscripts concerning qualitative genetics interpretation, gene symbols, and linkages should be sent to the Chairperson of the Soybean Genetics Committee for review. To facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol. "Manuscripts will not be reviewed for style except as this influences the clarity of interpretation. Manuscripts will not be "peer reviewed" unless requested by the author. Authors may submit unpolished (but comprehensible) manuscripts for review, unless peer review is requested. This should reduce delays involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of three weeks to return the reviewed manuscript to the Chairman (who will then return it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman of the Soybean Genetics Committee.

Assignment/Approval of Gene Symbols

If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Nelson is Curator for all maturity groups. A form for this purpose is on page 23 of this volume.

Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases.) The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is not necessary.

Committee Report

At the 1994 meeting, the SGC unanimously voted to recommend the following policy to Crop Science and to the USDA relative to the registration of soybean mapping populations:

"That soybean populations used for genetic mapping be registered in Crop Science with the understanding that the registering institution maintain the population for five years after which the Soybean Crop Advisory Committee will determine if the population will be maintained by the USDA Soybean Germplasm Collection."

Drs. J. E. Specht and J. H. Orf were asked to forward this recommendation to

CSSA and report the response.

Dr. Orf reported the results of the inquiry to CSSA as follows: The Crop Registration Committee did not reach a decision regarding the registration of mapping populations. Rather, it was decided to form a committee to consider the question. Dr. Orf will chair this committee which will include Dr. Roger Boerma as well as scientists working with wheat, oats, maize, and barley.

Related to this issue, Dr. Orf reported that the Soybean Crop Advisory Committee (SCAC) is now considering a system to incorporate mapping populations into the USDA Soybean Germplasm Collection. This system will probably function as follows:

1. When a request is made to put a mapping population into the collection, the SCAC will review the request to determine if the population is sufficiently valuable to merit inclusion. Five years after the initial decision to include the population, the SCAC will re-evaluate their initial decision to determine if the population should continue to be maintained.

2. The scientist requesting the inclusion of a mapping population in the Germplasm Collection will supply all available mapping data to all those who request it.

3. The institution of the originating scientist will be responsible for the distribution of seed of the population for five years. The institution will be obligated to supply the names of those requesting seed to the SCAC.

4. The SCAC is also considering the possibility of creating a special class of germplasm that could be disposed of after some defined period of time. Mapping populations would be included in this special class.

NEW BUSINESS:

1. Updating of the "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols" and the "Rules for Genetic Symbols":

   a. At the 1994 meeting of the SGC, the following motion passed unanimously: "Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing."

      A motion was made and passed unanimously that this statement be included in the introductory paragraph to the "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols".

   b. At the request of Dr. R. G. Palmer, the SGN will undertake a revision of the "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols" and the "Rules for Genetic Symbols". Both documents in their revised form are included in this issue of the Soybean Genetics Newsletter.

2. Gene symbols in perennial wild Glycine species: After consultation with Dr. Ted Hymowitz, a motion was made and unanimously passed that "Gene symbols in the wild perennial Glycine species be assigned based upon the existing "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols" and the "Rules for Genetic Symbols."

3. Election of new SGC Chairperson - February 1995 - February 1996: Dr. T. W. Pfeiffer was elected chairperson. All future correspondence regarding SGC business should be addressed to Dr. Pfeiffer.

Summary of Gene Symbols Approved During the Past Year

Five soybean gene symbols were approved by the Committee during the year ending February 1995, and are given in Table 1.

Table 1. Gene symbols approved March, 1994-February, 1995.

Date	Authors	Trait	Gene/Linkage
Oct. 6, 1994	Foley, Nelson, and Nickell	Dwarf plant	*Df7 df7* *Df8 df8*
Oct. 14, 1994	Ma, Chen, Buss, and Tolin	Soybean Mosaic Virus Resistance	*Rsv1-s*
Oct. 27, 1994	Ray, Hinson, Mankono, and Malo	Long juvenile trait	*J j*
Feb. 28, 1995	Harper and Nickell	hypernodulation	Rj7 rj7

Guidelines on the Evidence Necessary for the Assignment of Gene Symbols

Researchers are strongly encouraged to send all gene symbols and genetic interpretations to the Soybean Genetics Committee for review prior to publication to avoid duplication and/or confusion. Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing.

The following is a set of guidelines prepared by the Soybean Genetics Committee and intended to help researchers undertaking genetic analysis of soybean traits. Of necessity, these procedures will often need to be modified by the researcher to fit the specific situation, but an application of these guidelines should aid in making the correct genetic interpretation.

1. A genetic hypothesis is made on the basis of classification of segregating progeny, usually the F_2 generation and here called the hypothesis generation.

2. A second generation with a pedigree trace to the first generation, is classified to confirm the proposed genetic hypothesis. This second generation may be progeny of the hypothesis generation (usually F_3) or progeny of a testcross (F_1 x recessive homozygote).

3. Traits that are strongly influenced by nongenetic factors require verification of the classification scheme by evaluation of the progeny from homozygous plants of the hypothesis generation. Testcross data are not suitable for this purpose.

4. For genes controlling a phenotypic expression similar to that of previously published genes, data must be obtained to test for uniqueness and allelism. This will usually require crossing a homozygous line carrying the newly identified gene with the original sources of the previously published genes. If appropriate allelism tests are not included in a manuscript, the committee will request such information from the researcher. Molecular linkages can also be used to demonstrate that the allelism test conducted is the only one needed.

5. Identification of cytoplasmic factors requires reciprocal crosses between parents differing in the trait of interest. Since these factors are transmitted through the cytoplasm, the trait is expected to be associated only with the maternal parent in the F_1 and succeeding generations. Maternal effects need to be distinguished from cytoplasmic effects by using reciprocal F_1 and F_2 data.

6. Conclusive evidence for cytoplasmic factors should rule out self pollinations and nongenetic factors associated with the maternal parent. Selecting parents for reciprocal crosses that differ in nuclear genetic traits (e.g., flower or pubescence color) in addition to possible cytoplasmic traits will provide

evidence of cross- rather than self-pollinations by observed segregation for the nuclear genetic trait in succeeding generations.

7. Inheritance patterns in a hypothesis generation (F_2) and a confirming generation (F_3) are absolute requirements for differentiating between cytoplasmic factors and nuclear genetic traits.

8. Follow the guidelines (Rules for Genetic Symbols) published in the Soybean Genetics Newsletter to assign the symbol.

9. Submit the manuscript to the chair, Soybean Genetics Committee, for review of the genetic interpretation and approval of the gene symbol (see Soybean Genetics Newsletter for name and address). Please indicate in unequivocal terms your willingness to provide seed for allelism tests requested by researchers discovering genes with a similar phenotype. This does not restrict your asking for a signature on a Material Transfer Agreement.

10. If the line in which the new gene occurs in not already in the USDA Germplasm Collection, you are strongly encouraged to send a seed sample of the line to the curator of the Genetic Type Collection for assignment of a T-number and maintenance of the seed (see the current Soybean Genetics Newsletter for name and address).

References

Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombinational values in heredity. Hilgardia 24:235-278.

Hanson, W. D. 1959. Minimum family size for the planning of genetic experiments. Agron. J. 51:711-715.

Immer, F. R. 1930. Formulae and tables for calculating linkage intensities. Genetics 15:81-98.

Immer, F. R. and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-410.

Mather, K. 1951. The measurement of linkage in heredity. Methuen and Co., Ltd. London. John Wiley and Sons, Inc. New York.

Sedcole, J. R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Rules for Genetic Symbols

I. Gene Symbols

a. Gene symbols will not be assigned to traits for which no inheritance data are presented.

b. A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below. Gene symbols may, however, be written on one line.

c. Genes that are allelic shall be symbolized with the same base letter(s) so that each genetic locus will be designated by a characteristic symbol base.

d. Gene pairs that govern the same phenotype (including duplicate, complementary or polymorphic genes) constitute multiple loci that should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: Y_1, Y_2, etc.) The numerals may be written on the same line as the base. (Example: *Y1, Y2*, etc.) This shall be the only use of numerals. Letter designations should not be used. The numeral 1 is automatically a part of the first reported gene symbol for each base but may be omitted only until the second symbol is assigned.

e. The first pair of alleles reported for a genetic locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: *Ab, ab*; *Ab* is allelic and dominant to *ab*.)

f. If two alleles are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion and the alleles may be differentiated by adding one or two uncapitalized letters as superscripts to the base. When more than two alleles exist for a locus, the additional alleles, or those symbolized subsequently to the pair first published, shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: R, r^m, r.) This shall be the only use of superscripts. The letters may be written on the same line as the base if preceded by a hyphen. (Example: *Rps1-b, Rps1-k*, and *Ap-a, Ap-b, Ap-c*.) The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The letters may be an abbreviation of a descriptive term.

If independent mutations with the same or similar phenotype are identified at the same locus, until it is possible genetically to ascertain if they represent identical or separate alleles, the gene symbol should be followed by an identifying designation in parentheses. The identifying designation, which should **NOT** be in italics or underlined, can be the place where the mutation was found, the cultivar in which it was found, or any other relevant characteristic of the mutation. (Example: *ms1* [Tonica],

or *ms1* [Ames 2].) This will ensure that possible subtle differences between the mutations, such as differences in DNA sequence, or unique pleiotropic side effects, are not overlooked by workers using those genes.

g. Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.

h. The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clearcut. The decision for intermediate cases is at the discretion of the author, but should be in accordance with previous practices for the particular type of trait.

i. An underscore may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A_ represents both AA and Aa.)

j. A question mark may be used in place of a symbol when the locus or allele is unknown or doubtful. The name of the line in which the gene was identified should be included in the symbol, in parentheses. A hyphen preceding the question mark indicates an unknown allele at a known locus, the absence of a hyphen indicates an unknown locus. (Example: *Rps?* [Harosoy] an allele in Harosoy at an unknown locus or *Ap-?* [T160] an unknown allele in T160 at the *Ap* locus.)

k. Plus (+) symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II. Isoenzyme Symbols and Protein Gene Symbols

The following set of guidelines is to be used when assigning gene symbols to isoenzyme variants. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

a. A gene symbol (generally three letters) that indicates, as clearly as possible, the name of the enzyme should be used. (Example: Adh [alcohol dehydrogenase], Idh [isocitrate dehydrogenase].) The appropriate Enzyme Commission name and number should be used in the original article, when appropriate, to designate the specific enzyme activity being investigated.

b. The electrophoretic conditions used to characterize a locus or allele should be specified clearly and in sufficient detail to be repeated by others

interested in using the locus in genetic studies. The electrophoretic mobility, or other properties of an allele, should be clearly described by the authors.

c. Publications should include a photograph and/or an interpretive zymogram that allows readers to visualize the variability described by the authors, as well as to ensure that subsequent work corresponds to the original study.

III. Probe detected loci

The following guidelines are to be used for assigning locus names to probe-detected (RFLP) loci. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., IaSU). The prefix shall not be necessary in publications except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix is followed by a string of letters and/or integers that identify the probe used to detect the locus by the originating laboratory. This probe-identifying string should be limited to no more than six characters. This string should be separated from the prefix by a hyphen. (Example: IaSU-B317).

c. The probe-identifying sting is followed by the restriction endonuclease used in the restriction digest of the soybean genomic DNA that was probed. The following abbreviations for restriction enzymes are recommended: EcoRI = I, EcoRV = V, HinddIII = H,DraI = D, RsaI = R, Bc1I = B, TaqI = T. (Example: IaSU-B317I, IaSU-B317T).

d. Duplicate loci detected by the same probe should be identified with the same letter and integer base differentiated by integers (1, 2, 3, 4, etc.) consecutively assigned in the order of publication. These numerals are to be separated from the base string by a hyphen. Example: IaSU-B317I-1, IaSU-B317T-2, etc.)

e. Upon publication of new RFLP loci, researchers are strongly encouraged to
   1. make the probe identifying the locus/loci publicly available
   2. make available the identity of the restriction endonuclease used to generate the mapped polymorphism
   3. make available the identity of the genetic stock used to map the locus/loci
   4. make available the molecular weights of the polymorphic fragments used to map the locus/loci

IV. Random Amplified Polymorphic DNA (RAPD) loci

The following guidelines are to be used in assigning names to loci that are mapped using RAPD technology. The system adopted here is that which is generally employed in other species in which RAPD loci have been mapped.

a. Locus designations should begin with a letter identifying the origin of the primer. (Example: Operon Technologies, O)

b. The origin of the primer is followed by the primer name. (Example: Primer number 14 from Operon Technologies kit A, OA14.)

c. The primer name is followed in subscript by the fragment size in base pairs of the amplified fragment that is being mapped. (Example: An 800 bp fragment amplified with Operon Technologies primer 14 from kit A, $OA14_{800}$).

V. Simple sequence repeat (SSR) or microsatellite loci

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., IaSU). The prefix shall not be necessary in publications except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix should be followed by a string of letters that identify the core nucleotide repeat of the SSR followed by an identifying number. This string should be separated from the prefix by a hyphen and should not exceed eight characters. (Example: IaSU-at275, BARC-gata3412).

c. Upon publication of new SSR loci, researchers are strongly encouraged to
   1. make available the oligonucleotide primers sequences required for amplification of the SSR
   2. make available the identity of the genetic stock used to map the locus/loci

VI. Linkage and Chromosome Symbols

a. Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.

b. Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote

translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv, inversions; and Tri, primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second Def 1b, etc. The first published inversion in chromosome 1 shall be designated with the Arabic numeral that corresponds to its respective linkage group number.

c. Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2 the second case, etc. The first published deficiency shall be symbolized as Def A, the Def B, etc. The first published inversion shall be symbolized as Inv A, and the second as Inv B, etc. The first published trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

VII. Cytoplasmic Factor Symbols

a. Cytoplasmic factors shall be designated with one or more letters prefixed by *cyt-*. (Example: *cyt-G* indicates the cytoplasmic factor for maternal green cotyledons, *cyt-Y* indicates that for maternal yellow cotyledons.)

b. Designations for specific cytoplasmic factors following *cyt-*, shall follow the same format as for gene symbols. Base letters chosen to indicate apparent relationships among traits will have common initial letters for all loci in a related group of traits. Initial letters will be consistent with initial letters designating nuclear gene traits. (Example: *cyt-G* green seed embryo, *cyt-Y2* yellow leaves, becoming yellowish green.)

VIII. Priority and Validity of Symbols

a. A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.

b. In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

IX. Rule changes

a. These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date: _____ T number (assigned by curator) _____

Submitted by: _____

Address: _____

_____

_____

_____

_____

_____

_____

Return to:
R.L. Nelson, curator
USDA Soybean Germplasm Collection
Department of Agronomy
University of Illinois
1102 South Goodwin Avenue
Urbana, Illinois, 61801, U.S.A.

Strain Designation: _____

Genotype: _____

Phenotype: _____

_____

Parental Origin: _____

_____

_____

_____

When and where found and by whom: _____

_____

_____

_____

Description

Maturity Group _____ Stem termination _____

Pubescence color _____ Pubescence type and density _____

Seed coat luster and color _____ Hilum color _____

Flower color _____ other _____

Pod color _____ _____

Special instructions for growing or maintenance, if any: _____

_____

Literature References: _____

_____

_____

(List the reference(s) that first and best describe the discovery and inheritance of the trait. Please send relevant reprints to the curator.

Date seedlot received at Urbana: _____ Date T number assigned: _____

Institute of Crop Breeding and Cultivation[1]
Institute of Crop Germplasm Resources[2]
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The preliminary analysis of isoflavone content in Chinese soybean cultivars

Soybean seeds contain isoflavones which are responsible for the undesirable flavors as bitterness and astringency. Isoflavones have also been reported to exist in biological activities and biochemical activities, such as anti fungal and anti oxidant, etc. Here 50 soybean cultivars were examined by HPLC and obtained 3 low isoflavone cultivars and 3 high isoflavone cultivars of soybean.

Soybean seeds are well known as a very good food and as industrial materials with high protein and oil content. Soybean seeds and products often exist with various undesirable flavors such as green bean flavor, bitterness and astringency, etc. Green bean flavor mainly is brought out by activities of Lipoxygenase (Loxs) and elimination may occur through cross breeding and gamma-ray mutant breeding (Hajika et al., 1991; Ding An Lin et al., 1994). Saponin and isoflavones were considered as a cause of flavors such as bitterness and astringency in soybean seeds. Although most saponin content may be rid of by de-embryo treatment (Okudo et al., 1983) The isoflavone contained in cotyledons remained as a chief material which causes the bitter and astringent flavor (Kudou et al., 1991).

Soybean isoflavones are considered to have certain biological and biochemical activities (Akiyama et al., 1987; Coward et al., 1993) such as anti tumors and hemolysis effects, etc., (Naim et al., 1976) which may be greatly beneficial to the human health.

Japanese professor, K. Kitamura et al., (1991) has screened out soybean cultivars with low level isoflavones (Kitamura et al., 1991) and Brazilian Carro-panizzi also has one-cultivar (BR-36) with low level isoflavone and one (IAC-100) with high level isoflavone and anti-pest effect (Carrao-pannizzi and Kitamura, 1994). China is rich in soybean germplasm resources, but there is no research on the isoflavones of Chinese soybeans reported up to now, so it is necessary to screen various levels of Chinese soybeans for the use in the future.

In this paper 50 soybean cultivars from different production areas of China were sampled for determination of isoflavone contents. Three low and three high level varieties of isoflavone were found separately, which can be used in soybean breeding as parents to make new elites of soybean with various isoflavone levels, or used directly in other research areas.

Materials and Methods

1. Materials
    1) Cultivars: 50 soybean varieties [Glycine max (L.) Merr.] were studied by the Institute of Crop Germplasm Resources, CAAS. Among them, 13 are northeast spring planted soybeans; 19 are Huang Huai summer planted soybeans; 18 are southern multiple season planted soybeans (6 spring, 9 summer and 3 autumn planting).
    2) Isoflavone standard: daidzin and genistin were sent by Dr. K. Kitamura, Legume Lab, NARC, Japan.

2. Methods
    1) Preparation of soybean isoflavone extracts
    100 mg 60 mesh ground soybean flour was extracted with 80% ethanol at room temperature 1-2 hr. The supernatant was obtained by filtering or centrifuging. After that, the extract were used for analysis of isoflavones.
    2) Determination of soybean isoflavones
    Soybean isoflavones were measured by HPLC. The equipment used was a Shimadzu LC-6A with UV SPD-6AV detector and integrator. The column used was a (3.9 x 300 mm) μ-Bondapak TMC-18. The eluent consisted of 10-50% acetonitrile/water 0.I % acetic acid in a linear gradient from 0-55%. The temperature of the column was 40 °C and the flow rate was 1 μl/min, the injected volume was 10 μl and detections took place at UV 254 nm.
    3) Calculation of soybean isoflavone
    The identification of isoflavones was determined from purified daidzin and genistin standards (from Dr. K. Kitamura). The calculation was carried out by peak areas.

Results and Discussion

50 Chinese cultivars were analyzed by HPLC and discovered 3 high isoflavone cultivars and 3 low ones (Table 1). The peak patterns were estimated. There are 4 major related peaks: peaks 1, 2, 3 and 4 (Fig. 1). Peak 1 and 3 were identified as daidzin and genistin by standards; peak 2 and 4 were considered as malonyldaidzin and malonylgenistin according to Kudon (1991), but it is possible to have peak shift differences due to various conditions of analysis.

Table 1. 3 high and 3 Low isoflavone Chinese cultivars

Cultivar	Isoflavone Content (mg/100 g)				Productive areas
	Total	Daidzin	Genistin	D + G	
Chu Xiu	45.57	4.54	10.22	14.76	Huang Hai
Nan Hui Zao	84.55	11.29	17.17	28.46	South China
Hei Dou					
He Bao Dou	92.77	13.20	22.25	35.44	South China
Zhang Jia Kou	785.49	31.50	102.07	133.57	Huang Hai
Hei Dou					
Huai Dou No. 1	667.67	38.05	103.82	141.86	Huang Hai
Ji Lin No. 3	615.51	29.98	60.98	90.96	Northeast

A reversed-phase HPLC chromatograms of extracts from three Chinese soybean cultivars are given here in Fig 1. Peak A was from Zhang Jia Kou Hei Dou showed highest peaks; peaks B and C were from Chu Xiu and He Bao Dou presented lowest peaks. The peak area and isoflavone values between cultivars were quite different. The peak area in Zhang Jia Kou Hei Dou was 17 times that in the peak areain Chu Xiu and 8.5 times that in He Bao Dou. Generally speaking, the isoflavone values were observed with the following characteristics:

1. The difference of isoflavone contents between cultivars

Isoflavone contents showed a distinct difference among 50 cultivars. For most

cultivars, isoflavone contents were in 200-300 mg/ 100 g amount to 44% of the all cultivars observed. The lowest value was 45.57 mg/100 g; the highest value was 785.49 mg/100 g. Comparing our results to those in K. Kitamura's with a calculation of the sum of 1-4 peak areas, the lowest value in Chu Xiu (45.57 mg/100 g) was 10.52 mg/100 g less than that in Higomusume (55.09 mg/100 g); the highest value in Zhang Jia Kou Hei Dou (655.78 mg/100 g) was 161.26 mg/100 g higher than that in Lee (494.53 mg/100 g) (Table 2).

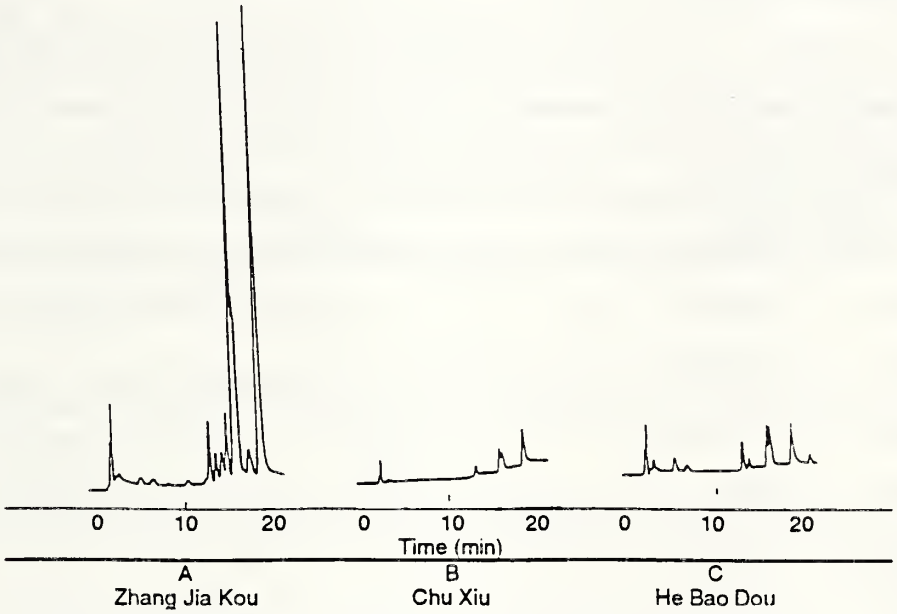

Figure 1. HPLC Chromatogram of isoflavones for three soybean cultivars

Table 2. Comparison of the isoflavone content between Chinese and Japanese soybean cultivars

Chinese cultivars	Chu xiu	He Bao Dou	Nan Hui Zao Hei Dou	Zhang Jia Kou He Dou	Huai Dou No. 1	Ji Lin No. 3
Isoflavone content (mg/100 g)	45.57	82.36	75.03	655.78	589.29	547.91
Japanese cultivars	Higomusumre	Kairyousirome	Kogonedaizu	Suzuyutaka	Lee*	
Isoflavone content (mg/100 g)	56.09	104.89	141.86	361.48	494.53	

Note: Data calculated by a sum of 14 areas
* U. S. cultivar

Among 50 determined Chinese cultivars the difference in isoflavone content is remarkable. It seems that in larger number of samples more cultivars may be found with much lower and higher isoflavone content.

Table 3. The relationships among isoflavone content and several parameters

Content of isoflavone (mg/100 g)		< 100	100-200	200-300	300-400	400-500	500-600	> 600	Total
Numbers tested		3	8	22	5	5	4	3	50
%		6	16	44	10	10	8	6	100
Spring sowing		1	3	11	3	3	3	1	25
Summer sowing		2	4	9	1	2	1	2	21
Autumn sowing			1	2	1				4
Color of seed coat	yellow	2	3	14	4	5	3	2	33
	green		4	3	1				8
	black	1	1	3				1	6
	brown			2			1		3
Product area	northeast		1	6	2	2	1	1	13
	huanghuai	1	1	7	2	3	3	2	19
	south	2	6	9	1				18

2. The isoflavone content with their distribution of the location in south China

The isoflavone content changed from 84.55 to 322.22 mg/100 g, the average was 189.90 mg/100 g. Among the 3 lowest cultivars of 50 tested cultivars, two were from Shanghai area; although another was from Huang Huai area, its breeding aim with Chu Xiu was vegetable use and exportation. It can be taken as an exception. In Huang Huai area, the isoflavone content varied from 115.52 to 785.49 mg/100 g, the average was 390.23 mg/100 g. The isoflavone content differed from 121.01 to 615.51 mg/100 g; the average was 332.91 mg/100 g in northeast and north Spring planting area. Generally, the content of isoflavone seems lower in the south than that in northern spring planting areas or Huang Huai summer planting areas. The reason

can be that people from south China like eating green vegetable soybean and keeping selection for long period naturally and artificially. This vegetable soybean had such characters as big seeds, easy cooking and good tasting. Some vegetable cultivars not only have lower isoflavone content but also with versatile Lox types. On the other hand, it may be related with environmental conditions such as special temperature and humidity in productive areas. Further observations are still needed.

3. The relationships between isoflavone content, seed size, and color of seed coat.

1) Size of seeds

Isoflavone content increased as seed size decreased. Large seeded cultivars often have low isoflavone levels and little difference between them, than cultivars with small seeds. It can be considered that isoflavone exists more in hypocotyl than that in cotyledon. For cultivars with big seeds, the volume is less share of proportion in hypocotyl than that in cotyledon, so the isoflavone level shows decrease and conversely for the small seeds (Table 4).

Table 4. The soybean isoflavones related with seed size and color of seed coat

Seed types	100 seed wt (g)	Numbers	Percent (%)	X	S	CV %
Big seed	>25	8	16	180.88	97.99	54.17
Med. seed	15-25	30	60	282.22	136.13	48.24
Small seed	>15	12	24	394.97	217.58	55.08
Color of coat						
Yellow	-	33	66	316.51	158.42	50.05
Green	-	8	16	199.58	92.57	46.38
Black	-	6	12	288.05	249.54	86.63
Brown	-	3	6	365.50	171.33	40.87

2) Color of seed coat, flower and pubescence

Soybean cultivars with different seed coat colors vary in levels of isoflavone. Green coat seeds seems to have lower levels of isoflavones, whereas brown and

black coat seed have higher levels (Table 4). This may be related to the metabolism of producing isoflavone and pigmentation, and may also be in the utilization of green seed coat varieties used mainly as vegetables. There were no differences seen between isoflavone levels and variable color of flowers, or pubescence.

4. The relationships between isoflavone levels and protein or oil content

Isoflavones seem to have a negative relationship with protein content and positive with oil content. However the tendency appeared insignificant, and large sample random tests as needed for getting further objective regulation.

Total results predicated that isoflavone content was low in soybean cultivars from southern of China but high in cultivars from Huang Huai summer planting productive area. It was believed that soybeans with much lower and higher isoflavone content will be discovered in a few years by analysis of larger samples. This will which can provide breeders with more special quality germplasm as parents for creating new high yielding and better tasting cultivars.

Reference

Akiyama, T., J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, and Y. Fukami. 1987. J. Biol. Chem. 262:5592.

Car rao-pannizzi, M.C.C. and K. Kitamura. 1994. World Soybean Research Conference V. Abstracts 20-26 February, 48.

Coward, L., N.C. Barnes, K.D.R. Setchell, and S. Barnes. 1993. J. Agric. Food Chem. 41:1961-1967.

Ding An Lin et al.. 1994. ACTA AGRONOMICA SINICA. 20(3):373-374.

Hajika, M., K. Igita, and K. Kitamura. 1991. Japan. J. Breed. 41:507-509.

Kitamura, K., K. Iqita, A. Kikuchi, S. Kudon, and K. Okubo. 1991. Japan. J. Breed. 41:651-654.

Kudou, S., Y. Fleury, D. Welti, D. Magnolato, T. Uchida, K. Kitamura, and K. Okubo. 1991. Agric. Biol. Chem. 55(9):2227-2233.

Naim, M., B. Gestetner, S. Zilkah, Y. Birk, and A. Bondi. 1974. J. Agric. Food Chem. 22:806-810.

Naim, M., B. Gestetner, A. Bondi, and Y. Birk. 1976. J. Agric. Food Chem. 24:1174.

Okudo, K., Y. Kobayashi., and K. Takahashi. 1983. Up to Date Food Processing 18:16-22 (in Japanese).

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Screening for salt tolerance to soybean cultivars of the United States

There are 0.61 billion ha. of salinized land in the world, of which about 20 million ha. are distributed in China. The salinization and sub-salinization of soil becomes the more serious problem in food production due to sea level rising and temperature moving up around the world. With population growth and industrial development, many fields are used as crop production, housing, and commications, which makes the amount of available land. On the other hand, people need more and more land to plant crops to fulfill the requirements of a growing population. In order to meet it's demand, there is a strategy to screen and develop salt tolerance soybean varieties for use in salinized soils and extension crop planting areas.

Materials and Methods

The experiment was conducted in a field of salinized soil located in Chanyi Farm of Laizhou gulf in Shandong province, China, which has dry and semi-dry monsoon climate. Annual average precipitation was 650 mm. The soil of the experiment field was a sandy loam with middle degree salinization, it has a strong osmosic characteristic that will be favorable toward maintaining stable soil salt content. The salt nursery was salinized by irrigating with a mixture of fresh water and subterranean saline water, which has the same ingredients as sea water. The soil is rich in potassium, insufficient in nitrogen and phosphorus, and lacks organic substance. The soil pH was 7.5.

The materials were evaluated directly in the field. The seeds were sown between June 25 to July 2. For each variety 30 seeds were sown in a 2 meter long row. Three replications were made in a random arrangement.

Before planting, the field was irrigated with fresh water to give soybeans a complete stand and provide vigorous early growth. Then soybean plants in V_2 or V_3 growth stage were irrigated with 15-17 ds/m saline water, a mixture of fresh water and underground salt water, until maintaining 3 cm water layer in the field. Symptoms of salt

stress injury on the leaves will appear after 3 to 5 days of irrigating. Evaluation results were recorded according to classification standards (Table 1). The final evaluation was made based on the mean of the three replications.

The experiment was conducted in 1986, 1987, 1989, and 1993, respectively. 257 varieties which are parts of old and new varieties introduced from the United States before 1984, were tested in 1986, among them only 75 varieties survived and retested in 1987. In 1989, 159 lines from the above 257 varieties were conducted as replication test, 48 lines were recored as salt tolerance varieties and retested in 1993.

Table 1. Evaluation classification standard of soybean tolerance in the field

Scale of salt tolerance	Symptom
1	Plant is normal with green leaf without dead plant. Only leaves in lower part of plant appear with mild symptoms.
2	Plant growth is good on the whole with less 30% plants appearing necrotic spot or slight curve on the leaves of lower part of plant.
3	Plant growth is stunted with 50% plants dead, most of leaves appear necrotic spot and curve.
4	Plant stop growing with less 85% plants dead, most leaves show necrosis and curve, only top 1 to 2 leaves remain green.
5	Plant stops growing with more than 85% plants dead. Only heart leaf remain green.

Results

The 257 accessions of USDA soybean germplasm collection were evaluated on salt tolerance. The results indicated that 71 accessions, 29.6% of genotypes tested, were very sensitive (5 degrees); 115 accessions, 44.7% of genotypes tested were sensitive (4 degrees); 56 accessions, 21.8% of genotypes tested, were middle tolerance varieties (3 degrees); 10 accessions, 3.9% of genotypes tested, (2 degrees) were tolerant varieties (Table 2).

Ten salt tolerant varieties were screened out from all of genotypes tested. They were Altona (00); Soysota and Tortoise Egg (I); Manchu (Hudson) (II); Cloud, Guelph and Mansoy (III); Morse, Pershing and Miles (IV) respectively. Among these varieties, 6

accessions are yellow seed coat, 2 lines are green seed coat, 1 line is black seed coat 1 line is brown seed coat. 3 accessions were improved varieties from the United States; 4 lines originate in China; 2 from Japan and 1 from Italy. The results are listed in Table 3.

Table 2. The evaluation of results of soybean salt tolerance in the field

Maturity	Scale				
Group	1	2	3	4	5	Total
000			1		1	2
00		1	8	3	3	15
0			2	14	6	22
I		2	13	17	12	44
II		1	12	31	16	60
III		3	9	30	18	60
IV		3	11	20	20	54
Total		10	56	115	76	257

Table 3. The results of evaluation of U. S. soybean varieties for salt tolerance in China

WDD No.	Variety	MG	Scale
0344	Sioux	OOO	5
0345	Agate	OO	4
0346	Flambeau	OO	4
0347	Manitoba Brown	OO	3
0348	Ogemaw	OO	3
0349	Pagoda	OO	5
0350	Capital	O	4
0352	Goldsoy	O	4
0353	Kabott	O	4
0354	Mandarin (Ottawa)	O	5
0355	Minsoy	O	5
0356	Poland Yellow	O	4

Table 3. - continued

WDD No.	Variety	MG	Scale
0357	Blackeye	I	5
0358	Burwell	I	3
0359	Cayuga	I	5
0360	Earlyana	I	3
0361	Elton	I	3
0362	Giant Green	I	4
0363	Habaro	I	4
0364	Hoosier	I	5
0365	Kagon	I	4
0366	Manchu Montreal	I	4
0367	Manchuria	I	5
0368	Mandarin	I	5
0369	Mandarin 507	I	4
0370	Medium Green	I	4
0371	Mendota	I	3
0372	Norsoy	I	4
0373	OAC 211	I	5
0374	Ontario	I	3
0375	Pautugal	I	4
0377	Soysota	I	2
0378	Tortoise Egg	I	2
0379	Wisconsin Black	I	3
0380	Aksarben	II	4
0381	Bansei	II	4
0383	Black Eyebrow	II	3
0385	Funman	II	3
0386	Goku	II	4
0387	Hakote	II	3
0388	Kanro	II	3
0389	Kanum	II	3
0391	Linman 533	II	5
0392	Manchu (Madison)	II	4
0393	Manchu (Hudson)	II	2

Table 3. - continued

WDD No.	Variety	MG	Scale
0394	Manchu 3	II	3
0395	Manchu 606	II	5
0396	Manchukota	II	4
0397	Mukden	II	4
0398	Richland	II	4
0399	Seneca	II	5
0400	Sousei	II	4
0401	Tastee	II	5
0402	Toku	II	4
0403	Waseda	II	4
0404	Wea	II	3
0405	Yellow Marvel	II	5
0407	Bavender Special A	III	3
0408	Bavender Special B	III	4
0409	Bavender Special C	III	3
0410	Chestnut	III	5
0411	Chusei	III	4
0412	Cloud	III	2
0413	Columbia	III	5
0414	Dunfield	III	3
0415	Fuji	III	4
0416	Granger	III	5
0417	Guelph	III	2
0418	Harman	III	4
0419	Illington	III	4
0420	Illini	III	3
0421	Ilsoy	III	3
0422	Jogun	III	4
0423	Jogun (Ames)	III	4
0424	Kura	III	4
0425	Lincoln	III	4
0426	Manchu	III	4
0427	Manchu (Lafayette)	III	4

Table 3. - continued

WDD No.	Variety	MG	Scale
0428	Manchu (Lafayette) B	III	4
0429	Manchu 2204	III	3
0430	Manchuria 13137	III	3
0431	Manchuria 20137	III	4
0432	Mandell	III	4
0433	Mansoy	III	2
0434	Miller 67	III	4
0436	Shingto	III	4
0437	Viking	III	4
0440	Wing Jet	III	4
0441	Wolverine	III	5
0442	A. K. (FC 30761)	IV	4
0443	A. K. (Kansas)	IV	5
0445	Boone	IV	5
0446	Chief	IV	3
0447	Ebony	IV	4
0448	Emperor	IV	4
0449	Funk Delicious	IV	3
0450	Gibson	IV	5
0453	Harbinsoy	IV	3
0454	Higan	IV	4
0455	Hokkaido	IV	3
0458	Imperial	IV	4
0459	Jefferson	IV	3
0461	Kingwa	IV	3
0462	Midwest	IV	3
0463	Morse	IV	2
0464	Norredo	IV	4
0465	Patoka	IV	3
0468	Sanga	IV	5
0469	Sato	IV	3
0470	Scioto	IV	5
0471	Shiro	IV	5

Table 3. - continued

WDD No.	Variety	MG	Scale
0472	Sooty	IV	5
0473	Virginia	IV	5
0474	Wilson	IV	5
0475	Wilson-5	IV	5
0476	Wilson-5 B	IV	5
0477	Wilson-6	IV	5
0478	Maple Presto	OOO	3
0479	Acme	OO	4
0480	Ada	OO	5
0481	Altona	OO	2
0482	Crest	OO	3
0483	Maple Amber	OO	3
0484	Maple Arrow	OO	3
0485	McCall	OO	4
0486	Morsoy	OO	3
0487	Norman	OO	3
0488	Portage	OO	3
0489	Chico	O	3
0490	Clay	O	3
0491	Comet	O	4
0492	Dawson	O	4
0493	Evans	O	4
0494	Grande	O	4
0495	Grant	O	5
0496	Hardome	O	4
0497	Merit	O	4
0498	Norchief	O	4
0499	Ozzie	O	5
0500	Sijpson	O	4
0501	Swift	O	5
0502	Traverse	O	4
0503	Vansoy	O	4
0504	Wilkin	O	5

Table 3. - continued

WDD No.	Variety	MG	Scale
0505	Anoka	I	5
0506	Blackhawk	I	5
0507	Chippewa	I	4
0508	Chippewa 64	I	4
0509	Coles	I	3
0510	Disoy	I	4
0511	Dunn	I	4
0512	Hardin	I	4
0513	Hark	I	4
0514	Harlon	I	4
0515	Harly	I	5
0516	Hodgson	I	5
0517	Hodgson 78	I	4
0518	Lakota	I	4
0519	Monroe	I	3
0520	Rampage	I	3
0521	Renville	I	3
0522	Steel	I	3
0523	Vinton	I	3
0524	Vinton 81	I	3
0525	Weber	I	5
0526	Wirth	I	4
0527	Amcor	II	4
0528	Amsoy	II	4
0529	Amsoy 71	II	5
0530	Beeson	II	4
0531	Beeson 80	II	5
0532	Century	II	4
0533	Century 84	II	4
0534	CN210	II	5
0535	CN290	II	5
0536	Corsoy	II	4
0537	Corsoy 79	II	5

Table 3. - continued

WDD No.	Variety	MG	Scale
0538	Elgin	II	5
0539	Gnome	II	4
0540	Hark	II	4
0541	Harcor	II	3
	Harosoy	II	3
0542	Harsoy 63	II	4
0543	Harwood	II	4
0544	Hawkeye	II	4
0545	Hawkeye 63	II	4
0546	Henry	II	3
0547	Keller	II	3
0548	Lindarin	II	4
0549	Lindarin 63	II	5
0550	Madison	II	4
0551	Magna	II	4
0552	Marion	II	5
0553	Miami	II	5
0554	Nebsoy	II	5
0555	Platte	II	5
0556	Prize	II	4
0557	Protana	II	4
0558	Provar	II	3
0559	Sloan	II	4
0560	Vickery	II	4
0561	Wells	II	4
0562	Wells 2	II	4
0563	Adams	III	3
0564	Adelphia	III	5
0565	BSR 201	III	4
0566	BSR 301	III	4
0567	Calland	III	4
0568	Cumberland	III	4
0569	Elf	III	5

Table 3. - continued

WDD No.	Variety	MG	Scale
0570	Fayette	III	5
0571	Ford	III	4
0572	Harper	III	4
0573	Hobbit	III	4
0574	Kanrich	III	4
0575	Kim	III	4
0576	Mead	III	5
0577	Oakland	III	5
0578	Pella	III	5
0579	Ross	III	5
0580	Shelby	III	5
0581	Sprite	III	5
0582	Verde	III	4
0583	Wayne	III	5
0584	Will	III	5
0585	Williams	III	4
0586	Williams 79	III	5
0587	Williams 82	III	5
0588	Winchester	III	4
0589	Woodworth	III	5
0590	Zane	III	3
0591	Bethel	IV	4
0592	Bonus	IV	5
0593	Clark 63	IV	5
0594	Columbus	IV	5
0595	Custer	IV	5
0596	Cutler	IV	4
0597	Cutler 71	IV	5
0598	Delmar	IV	4
0599	Desoto	IV	3
0600	Douglas	IV	5
0601	Egyptian	IV	4
0602	Flanklin	IV	5

Table 3. - continued

WDD No.	Variety	MG	Scale
0604	Kaikoo	IV	4
0606	Kent	IV	4
0607	Lawrence	IV	4
0608	Miles	IV	2
0610	Oksoy	IV	5
0612	Pershing	IV	2
0613	Pixie	IV	4
0614	Pomona	IV	4
0615	Scott	IV	4
0616	Sparks	IV	4
0617	Union	IV	4
0618	Wabash	IV	3
0619	Ware	IV	4
0620	Wye	IV	5

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Survey of leaf feeding insects on soybeans in Nanjing

Leaf-feeding insects are one of the major constraints to soybean yield and quality in China. Currently, insecticide application is the major way to control pests. However, it has caused serious environmental pollution, and also led to the resistance of insects to the chemicals, which resulted in the need for alternative control strategies.

In the last five years, cotton ballworm [Heliothis armigera (Hubner)] and soybean hawk moth [Clanis bilineata Walker] in Huanghe-Huaihe valleys, the soybean leaf folder [Lamprosema indicata (F.)], the soybean looper [Ascotis selenaria (Schiffermuller et Denis)], and the tobacco cutworm [Prodenia litura (F.)] in southern China have been found to be the major pests on soybeans. Ecologists and soybean breeders emphasized on the strategy to control defoliators through genetic improvement. A program of investigating leaf-feeding insects on soybeans and screening for sources of resistance has been carried out in Soybean Research Institute of Nanjing Agricultural University since 1983. This is a report of soybean leaf-feeding insects occuring in Nanjing, China.

Material and Methods

1. Lamp-Trapped Moths of Leaf-feeders

An electric trapping lamp was set in soybean field in Nanjing. The lamp was turned on at dusk and turned off at dawn during soybean growing season in 1983, 1984, 1992, 1993, and 1994. The trapped moths were identified and counted everyday. The accumulated numbers and the percentage of the major species of soybean leaf-feeders trapped are shown in Table 1.

2. Leaf-Feeder Species in Soybean Field

Field investigations were carried out in Nanjing during 1990-1994. Leaf feeding

insects were collected from soybean fields and identified in the lab. Except some rare species, the collected ones are listed in Table 2.

Table 1. The accumulated amounts and the percentage of the main defoliators trapped in trapping lamp (Nanjing, 1983,1984,1992,1993,1994)

Species*	Year and accumulated period					Sum	%
	07/15/83 09/15/83	07/15/84 -09/15/84	07/15/92 -09/15/92	09/01/93 -09/30/93	08/02/94 -09/03/94		
SBL	207	171	294	186	140	998	32.8
SLF	153	137	318	169	87	864	28.4
TCW	92	103	40	7	53	295	9.7
STM	71	88	46	19	35	259	8.5
TSP	45	32	77	30	63	247	8.1
SHM	44	55	22	--	--	121	4.0
Others	82	46	61	35	34	258	8.5
Total	694	632	858	446	412	3042	100.0

* Note: SBL = Soybean looper [Ascotis selenaria (Schiffermuller et Denis)]
SLF = Soybean leaf folder [Lamprosema indicata (Fabricius)]
TCW = Tobacco cutworm [Prodenia litura Fabricius]
STM = Soybean tussock moth [Cifuna locuples Walker]
TSP = Three-spotted plusia [Plusia agnata Staudinger]
SHM = Soybean hawk moth [Clanis bilineata Walker]
Others = Ilattia octo (Guenee), Bomolocha tristalis Lederer
Hypena taenialoides Chu et Chen, Mocis undata (Fabricius)

Table 2. A list of soybean leaf-feeders in Nanjing (1990-1994)

Order	Family	Species
Coleoptera	Chrysomelidae	Hactocnenva ingenua, Baly
Coleoptera	Chrysomelidae	Monolepta hieroglyphica, Motschulsky
Coleoptera	Chrysomelidae	Paraluperodes suturalis nigrobilineatus, Motschulsky
Coleoptera	Meloidae	Epicauta gorhami, Marseul
Coleoptera	Meloidae	Epicauta obscurocephala, Reitter
Coleoptera	Tenebrionidae	Opatrum subatum, Fald.

Table 2. continued

Order	Family	Species
Hemiptera	Coreidae	Anoplocnemis binotata, Distant
Hemiptera	Coreidae	Cletus trigonus, Thunberg
Hemiptera	Coreidae	Riptortus clavatus, Thunberg
Hemiptera	Coreidae	Riptortus pedestris, Fabricius
Hemiptera	Miridae	Adelphocoris saturalis, Jakovlev
Hemiptera	Pentatomidae	Dolycoris baccarum (Linnaeus)
Hemiptera	Pentatomidae	Eurydema dominulus (Scopoli)
Hemiptera	Pentatomidae	Nezara viridula (Linnaeus)
Hemiptera	Pentatomidae	Stollia guttiger (Thunberg)
Hemiptera	Plataspidae	Megacopta cribraria (Fabricius)
Homoptera	Aphididae	Aphis craccivora. Koch
Homoptera	Aphididae	Aphis glycines. Matsumura
Homoptera	Aphididae	Aphis gossypii. Glover
Homoptera	Cicadellidae	Empoasca flavescens (Fabricius)
Homoptera	Cicadellidae	Tettigoniella viridis Linnaeus
Lepidoptera	Arctiidae	Amsacta lactinea (Cramer)
Lepidoptera	Arctiidae	Spilarctia subcarnea. Walker
Lepidoptera	Cochlidiidae	Those sinensis (Walker)
Lepidoptera	Geomotridae	Calothysanis comptaria. Walker
Lepidoptera	Lymantriidae	Cifuna locuples. Walker
Lepidoptera	Noctuidae	Anomis flava. Fabricius
Lepidoptera	Noctuidae	Plusia agnata. Staudinger
Lepidoptera	Noctuidae	Ascotis selenaria (Schiffermuller et Denis)
Lepidoptera	Noctuidae	Bomolocha tristalis. Lederer
Lepidoptera	Noctuidae	Heliothis armigera (Hubner)
Lepidoptera	Noctuidae	Hypena rostralis. Linnaeus
Lepidoptera	Noctuidae	Hypena taenialoides. Chu et Chen
Lepidoptera	Noctuidae	Ilattia octo (Guenee)
Lepidoptera	Noctuidae	Mocis undata (Fabricius)
Lepidoptera	Noctuidae	Prodenia litura. Fabricius
Lepidoptera	Pieridae	Colias erate poliographus. Motschulsky
Lepidoptera	Pieridae	Colias hyale. Linnaeus
Lepidoptera	Pieridae	Vanessa cardui. Linnaeus

Table 2. continued

Order	Family	Species
Lepidoptera	Pyralidae	Lamprosema indicata (Fabricius)
Lepidoptera	Pyralidae	Udea ferrugalis (Hubner)
Lepidoptera	Sphingidae	Clanis bilineata. Walker
Orthoptera	Acrididae	Acrida cinerea. Thunberg
Orthoptera	Catantopidae	Chondracris rosea (De Geer)
Orthoptera	Catantopidae	Oxya chinensis (Thunberg)
Orthoptera	Pyrgomorphidae	Atractomorpha lata (Motschulsky)
Orthoptera	Pyrgomorphidae	Atractomorpha ambigua. Bolivar
Orthoptera	Oedipodidae	Locusta migratoria manilensis (Meyen)

3. The Dynamic Changes of the Major Leaf-Feeders

Further investigations were conducted in a fixed soybean field with 10 ha acreage during 1992-1994. In the successive three years, the field survey were conducted 10, 4 and 6 times, respectively. Each time, the leaf-feeding insects were collected from five 4 m^2 plots randomizedly sampled in the field, then identified and counted in lab. The main species and the sums of insects from five plots were shown in Table 3, Table 4, and Table 5.

Table 3. The amount and the percentage of defoliators collected from soybean field (number of insects from five 4 m^2 plots, Nanjing, 1992)

Species*	Date										Sum	%
	07/16	07/24	07/31	08/06	08/13	08/20	08/27	09/03	09/12	09/17		
SLF	9	37	257	53	80	392	476	373	38	2	1717	89.4
SBL	16	10	14	28	5	23	17	12	29	13	167	8.7
TCW	0	0	0	0	0	0	0	1	3	0	4	0.2
STM	0	0	0	4	4	0	1	2	1	0	12	0.6
SHM	0	1	3	2	1	1	8	1	0	0	17	0.9
TSP	0	0	0	0	0	0	0	1	0	2	3	0.2
Total	25	48	274	87	90	416	502	390	71	17	1920	100.0

* for abbreviation definitions see Table 1 Note

Table 4. The amount and the percentage of defoliators collected from soybean field (number of insects from five 4 m^2 plots, Nanjing, 1993)

Species*	Date				Sum	%
	09/01	09/08	09/16	09/23		
SLF	840	353	270	52	1515	92.7
SBL	24	16	31	3	74	4.5
Others**	12	8	20	6	46	2.8
Total	876	377	321	61	1635	100.0

*Note for abbreviation definitions see Table 1 Note
**Others Prodenia litura Fabricius, Cifuna locuples Walker, Plusia agnata Staudinger, Clanis bilineata Walker, Ilattia octo (Guenee), Bomolocha tristalis Lederer Hypena taenialoides Chu et Chen, Mocis undata (Fabricius)

Table 5. The amount and the percentage of defoliators collected from soybean field (number of insects from five 4 m^2 plots, Nanjing, 1994)

	Date							
Species*	08/16	08/31	09/06	09/16	09/27	10/07	Sum	%
TCW	22	74	167	293	188	46	790	54.8
SBL	7	89	111	146	72	28	453	31.4
SLF	18	21	14	5	7	0	65	4.5
TSP	25	29	3	8	0	0	65	4.5
STM	8	16	12	0	4	1	41	2.8
SHM	0	0	1	0	6	2	9	0.6
Others**	9	4	0	4	0	1	18	1.2
Total	89	233	308	456	277	78	1441	100.0

*Note for abbreviation definitions see Table 1 Note
**Others Ilattia octo (Guenee), Bomolocha tristalis Lederer Hypena taenialoides Chu et Chen, Mocis undata (Fabricius)

The data showed that the relative constituents of the leaf-feeder population in soybean field in Nanjing changed from year to year. During 1980's, the soybean leaf

folder was not as important as soybean looper and tobacco cutworm. But during 1992-1993, it became the most important one and accounted for about 90% among all defoliators, while soybean looper and tobacco cutworm accounted for only less than 10% (Table 3 and 4). However, in 1994, the situation was reversed (Table 5). The population of soybean leaf folder dropped down to about 5%, while that of soybean looper and tobaco cutworm rose up to 30% and 54%, respectively, in a total of about 85%.

Despite the dynamic changes of constituents of the leaf-feeder population, soybean leaf folder, tobacco cutworm, and soybean looper were considered to be the three most important leaf-feeding insects in Nanjing based on both the investigation of lamp-trapped moths and the survey of leaf-feeders in the field.

References

Liu, Tiruo (ed.). 1979. Soybean Diseases and Pests. Agricultural Publishing House, Beijing, China. (Chinese).

Liu, Zhen. 1991. Soybean hawk moth attack Xinxiang, Henan Province in 1990. Journal of Plant Protection (4):53-54. (Chinese).

Ma, Shijun et al. (ed.). 1983. An English-Chinese Glossary of Agro-entomology. Agricultural Publishing House, Beijing, China.

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Screening for resistant sources of soybeans to leaf-feeding insects

Three soybean genotypes, P.I. 171451, P.I. 227687 and P.I. 229358, have been screened out for their resistance to Mexican bean beetle [Epilachna varivestis Mulsant] (Van Duyn et al, 1971). Subsequent studies on the three P.I. s proved them to be resistant to diverse leaf-feeding insects including corn ear worm [Heliothis zea (Boddie)], tobaco bud worm [Heliothis virescens (F.)], cabbage looper [Trichoplusia ni Hubner], striped blister beetle [Epicauta vittata (F.)], soybean looper [Pseudoplusia includens (Walker)], and velvet bean caterpillar [Anticarsia gemmatalis Hubner] (Clark et al., 1972, Luedder and Dickerson, 1977, Hatchett et al., 1976, Kilen et al., 1977, and Beach 1988). Commercial soybean cultivars and advanced breeding lines were evaluated for their resistance to some insect species (Lambert and Kilen 1984; Joshi and Nobakht 1988). Several breeding lines with resistance to leaf-feeding insects were registered (Kilen and Lambert 1993). By incorporating resistance from P.I. 229358 and P.I. 171451 to commercial cultivars, Lamar and Crockett with multiple resistance to leaf-feeding insects were developed and released (Hartwig, Lambert and Kilen 1990, Bowers 1990).

Breeding for resistance to leaf-feeders has been recognized as one of the most important breeding objectives in southern China. P.I. 171451, P.I. 227687, and P.I. 229358 were introduced and evaluated for their resistance to the local leaf feeders in Nanjing. The resistance was shown to be good but there appeared some adaptation problem due to the inferior agronomic performance. Therefore, other sources of resistance are expected. This paper reports the results of screening for new resistant sources of soybeans to local leaf-feeding insects conducted in Nanjing, China.

Materials and Methods

Materials used in screening for sources of resistance to leaf-feeding nsects were a part of germplasm collections in Soybean Research Institute, Nanjing Agricultural

University. All the field-screening experiments were conducted in Nanjing. To use the natural inoculation of leaf-feeding insects, no insecticide was applied to soybeans in experiment field.

Visual scores of defoliation were made on each plot two or more times after flowering with a scale of 0 through 4.

0 = 50% or over 50% leaves with 0 to 5% defoliation
1 = 50% or over 50% leaves with 6 to 25% defoliation
2 = 50% or over 50% leaves with 26 to 50% defoliation
3 = 50% or over 50% leaves with 51 to 75% defoliation
4 = 50% or over 50% leaves with 76 to 100% defoliation

The visual scores were the comprehensive indicator of the defoliation due to the population of the constituent species of leaf-feeders which might change from year to year. Principal Component Analysis (PCA) was applied to combine the data from various times of records. The first principal component scores were used in the resistance grade system since they were dominant over the others and accounted for more than 70% of the total variation. Entries were grouped into ten categories according to the following grade system of the first principal component scores.

Grade 0	<= M-2.0S	high resistant
Grade 1	M-2.0S -- M-1.5S	high resistant
Grade 2	M-1.5S -- M-1.0S	resistant
Grade 3	M-1.0S -- M-0.5S	resistant
Grade 4	M-0.5S -- M-0.0S	moderate
Grade 5	M-0.0S -- M+0.5S	moderate
Grade 6	M+0.5S -- M+1.0S	susceptible
Grade 7	M+1.0S -- M+1.5S	susceptible
Grade 8	M+1.5S -- M+2.0S	high susceptible
Grade 9	>= M+2.0S	high susceptible

Here the M and S represent the mean and the standard deviation of the first principal component scores, respectively.

The primary screening was carried out in 1983 and 1989 with 3276 and 3143 different accessions, respectively, mostly from China and partly from abroad, in 3-row plots 3 m long and rows 0.5 m apart, without replication. From visual scores recorded at

about R_2 and R_5, 198 resistant and 29 susceptible genotypes from the experiment in 1983, 120 resistant and 24 susceptible genotypes from the experiment in 1989, and the three P.I. checks (P.I. 117451, P.I. 227687, and P.I. 229358) were chosen for the experiment in 1990.

In 1990, the selected 374 genotypes were planted in a randomized complete block design with 2 replications of 3-row plots, 2 m in length and 0.5 m apart. At four different times, visual scores were recorded during R_1 through R_6. The data from 364 genotypes with all four records were used in the principal component analysis. Among them, 51 comparatively resistant and 42 susceptible genotypes along with 3 P.I. checks were chosen for the experiment in 1992.

In 1992, the selected 96 genotypes were planted in a randomized complete block design with 6 replications of 3-row plots, 2 m long and 0.5 m apart. Visual scores were recorded four times during R_1 through R_6. Based on the principal component analysis, 18 comparatively resistant and 18 susceptible genotypes along with 3 P.I. checks were chosen for experiment in 1993.

In 1993, the 39 genotypes were planted in a randomized complete block design with 6 replications of 3-row plots, 2 m long and 0.5 m apart. Visual scores were recorded six times during R_1 through R_6.

In 1994, all the 39 genotypes tested in 1993 plus five others were tested again in a randomized complete block design with 6 replication of 3-row plots, 2 m long and 0.5 m apart. Visual scores were recorded six times during R_1 through R_6.

Results and Discussion

1. Distribution of Resistant Grade

The distribution of resistant grade in each testing year is shown in Table 1. Out of the 6419 genotypes evaluated in 1983 and 1989, 0.4% performed to be highly resistant, 8.2% resistant, 72.0% moderate, 18.5% susceptible, and 0.9% highly susceptible.

2. Resistant Genotypes

From the 1990-1993 experiments, six genotypes, N5454.3, N4029.3, N3697, N3018, N3400.1, and N1178.22, were screened out for their relatively high resistance to leaf-feeding insects. The former five genotypes constantly performed to be the least defoliated among the tested entries in 1990-1993, but in 1994, N1178.22 was the least

defoliated. The resistant grades of the sceeened genotypes and the three P.I. checks in different years were listed in Table 2.

Table 1. The distribution of resistant grade in each experimental year

Grade	1983	1989	1990	1992	1993	1994
0	0	4	4	0	0	0
1	0	21	12	8	1	1
2	11	108	38	10	7	3
3	104	304	59	16	6	15
4	712	540	81	13	6	6
5	1746	1621	68	16	4	6
6	463	471	46	15	6	6
7	212	44	28	13	6	6
8	28	9	14	3	3	0
9	0	21	14	2	0	2
Total	3276	3143	364	96	39	45

Table 2. The PCA grades of the sources of resistance

Genotype	1990	1992	1993	1994
N5454.3	0	1	1	3
N4029.3	0	1	2	6
N3697	2	1	2	3
N3018	1	1	2	5
N3400.1	2	1	2	6
N1178.22	1	3	3	1
P.I. 171451	3	5	4	4
P.I. 227687	1	2	3	3
P.I. 229358	4	4	4	3

Comparing with the six genotypes screened out from this study, the three P.I. checks performed with relatively low resistance (Table 2). Obviously, it is due to the different constituents of the insect population. Soybean looper [Ascotis selenaria (Schiffermuller et Denis)], soybean leaf folder [Lamprosemandicata (Fabricius)], and tobacco cutworm [Prodenia litura Fabricius] were the dominant insects on soybeans in Nanjing, while those important in the westen part of the world, such as Mexican been beetle, velvet bean caterpillar, soybean looper [Pseudoplusia includens (Walker)], corn ear worm, tobaco bud worm, cabbage looper, striped blister beetle, did not occur in Nanjing.

In the year of 1992 and 1993, the main leaf-feeding insects occuring in Nanjing were soybean leaf folder (about 90%), soybean looper (about 5%) and tobacco cutworm (less than 5%), but in 1994, the population structure of leaf-feeding insects was completely different. The tobacco cutworm accounted for 54.8%, the soybean looper 31.4%, and the soybean leaf folder 4.5%. This sharp change resulted in the differential resistant performence of the soybean genotypes in 1994 (Table 2). It could be inferred that the resistant mechanism of soybeans to soybean leaf folder was different from that to soybean looper and tobacco cutworm. Field observation indicated that genotypes resistant to soybean leaf folder had sparse, short and soft pubescences on the leaf surfaces, while genotypes susceptible to soybean leaf folder had dense, long and strong pubescenses on the leaf surfaces. It seemed that the resistance to soybean leaf folder was not related to the color, thickness and stiffness of the leaves.

References

Bowers, G.R., Jr. 1990. Registration of "Crockett" soybean. Crop Science 30(2): 427.

Hartwig, E.E, L. Larbert and T.C. Kilen. 1990. Registration of "Lamar" soybean. Crop Science 30(1):231.

Jushi, J.M. and M. Nobakht. 1988. Evaluation of commercial soybean cultivars and advance breeding lines for non-preference to Heliothis zea. Soybean Genetics Newsletter 15:124-126.

Kilen, T.C. and L. Lambert. 1993. Registration of three glabrous and three dense pubescent soybean germplasm lines susceptible (D88-5320, D88-5295), Moderately resistant (D88-5328, D885272), or resistant (D909216, D90- 9220) to foliar-feeding insects. Crop Science 33(1):215.

Lambert, L. and T.C. Kilen. 1984. Multiple insect resistance in several soybean genotypes. Crop Science 24:887-890.

Van Duyn, J. W. et al. 1971. Resistance in Soybeans to the Mexican Bean Beetle I: Source of Resistance. Crop Science. 11:572-573.

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A report on the nuclear cytoplasmic male sterility from a cross between two soybean cultivars

Although at least six patents related to hybrid soybeans have been granted, no commercial hybrid soybean cultivars have been released. So far, six nuclear male sterile genes, ms_1 through ms_6, have been described. Some of them have been successfully used in breeding programs, especially in recurrent selection for yield, protein content and oil content. This type of male sterility is hard to use in producing large quantity of hybrid seeds. Davis (1986) reported a route to produce F_1 hybrid soybean using a nuclear-cytoplasmic male sterile parent which incorporates cytoplasmic male sterility (from a Mandarin derived variety such as ELF) and the recessive fertility restorers r_1 (from a Dunfield derived variety such as Bedford) and the r_2 (from a Tokyo derived variety such as Braxton). Sun (1994) reported a nuclear-cytoplasmic male sterile line from interspecific crosses between Glycine soja and Glycine max. Peng (1994) mentioned a line carrying male sterile cytoplasm associated with a dominant nuclear gene, but not in details. This is a report of the nuclear-cytoplasmic male sterility from a cross between two soybean cultivars, N8855 and N2899.

Crosses and their performance of sterility

The cross N8855 X N2899 was first made in 1988. The following year, a part of the flowers of F_1 plants were artificially pollinated with another parent and the remaining flowers were pollinated naturally. An abnormal phenomenon was observed. Bearing on the F_1 plants, there were only a few pods which were basically from the artificial pollination. Segregation on sterility in their progenies was observed.

The reciprocal crosses, N8855 X N2899 and N2899 X N8855, were made in 1990. A large number of F_1 seeds from the crosses were obtained. Unfortunately, the F_1 plants were ruined by the heavy flood in 1991.

In the winter of 1991, the crosses, N8855 X N2899 and N2899 X N8855, were

made in Hainan Island, China. Due to severe insect damage, only one seed from N8855 X N2899 and nine seeds from N2899 X N8855 were obtained. The F_1 seeds were planted in Nanjing the next summer. As expected, the unique F_1 plant from N8855 X N2899 showed sterile producing six seeds, and the nine F_1 plants from N2899 X N8855 were all fertile each producing at least 60 seeds. The F_2 seeds from F_1 plants were sown in 1993. One of the six F_2 plants derived from the sterile F_1 plant kept sterile, the other five showed fertile. All of the F_2 plants derived from the nine fertile F_1 plants were still fertile. In 1994, seeds from each of the six F_2 plants of N8855 X N2899 were planted as $F_{2:3}$ families. The sterility segregation is shown in Table 1.

The reciprocal crosses, N8855 X N2899 and N2899 X N8855, were again made in 1992. Four seeds were obtained from each of the two crosses. In 1993, just the same as previous results, the four F_1 plants of N8855 X N2899 showed sterile, one of which did not set pod, and the four F_1 plants of N2899 X N8855 were fertile. In 1994, A segregation of 17 sterile to 10 fertile were observed in the F_2 of N8855 X N2899. No segregation was found in the F_2 population of N2899 X N8855 (Table 2).

Table 1. The sterility segregation of six $F_{2:3}$ families of N8855 x N2899

	$F_{2:3}$ family		
F_2 plant	No. sterile plants	No. fertile plants	Total
Plant 1, sterile	0	8	8
Plant 2, fertile	0	12	12
Plant 3, fertile	0	10	10
Plant 4, fertile	2	8	10
Plant 5, fertile	0	12	12
Plant 6, fertile	1	17	18

Character of the Sterile and Fertile Plants

Before flowering, it is difficult to distinguish the sterile plants from the fertile plants in F_2 generation. But after flowering, the leaves of the sterile plants became more green and thicker than those of fertile plants, and the stems became stronger with a few developed pods and a large number of undeveloped little podes on them.

Physiologically, there is no large difference between the date of seed maturity of sterile plants and that of fertile plants. The former was only a few days later than the latter. But for sterile plants, after seed matured, the leaves kept green till the frost. The number of pods per sterile plant was about 0-26, with an average of 14.8 (5.9 one-seeded, 6.0 two-seeded, and 2.9 three-seeded), while the fertile plants had an average of 118.1 pods per plant (30.7 one-seeded, 56.2 two-seeded and 31.2 three-seeded).

Pollen germination test of F_2 plants of N8855 X N2899 was carried out in 1994 according to Gai (1975). The germination rates of pollen grains from the sterile plants were 0-3.2%, while those of pollen grains from fertile plants were 37-86%. There is no significant difference in appearance observed between pollen grains from sterile plants and those from fertile plants.

Table 2. Sterility segregation in F_2 generation

		$F_{1:2}$ family	
Cross	F_1 plant	No. sterile plants	No. fertile plants
N8855 x N2899	Plant 1, sterile	1	4
	Plant 2, sterile	4	2
	Plant 3, sterile	12	4
	Plant 4, sterile	--	--
N2899 x N8855	Plant 1, fertile	0	67
	Plant 2, fertile	0	73
	Plant 3, fertile	0	67
	Plant 4, fertile	0	44

Conclusion and Discussion

According to the above observation, the following facts were true: (1) both N8855 and N2899 were fertile, and the F_1 of N8855 X N2899 was male sterile and female fertile, while the F_1 of N2899 X N8855 was both male and female fertile, (2) the male sterility was not due to translocation since there showed no stable half-sterility

phenomenon, (3) N8855 had cytoplasmic male sterile gene or genes, and N2899 had nuclear male sterile gene or genes, (4) the male sterility from N8855 X N2899 was conditioned by the joint effect of nuclear and cytoplasmic genes.

As for the nuclear gene system controlling the male sterility, there was not enough evidence to make conclusion. There appeared obvious discrepancy in F_1 being sterile in fact but fertile in expectation if male sterile allele was recessive. There appeared also obvious discrepancy in $F_{2:3}$ if male sterile allele was dominant since the $F_{2:3}$ family derived from sterile F_2 plant should be sterile or sterile+fertile in expectation but was completely fertile in fact, and the $F_{2:3}$ family derived from fertile F_2 plant should be completely fertile in expectation but was fertile+sterile in fact. It seemed that the observed data could not be explained very well by single dominant or recessive allele. A complicated nuclear gene system for this kind of male sterility might be existed. It will be remained for further study. Maybe the process of finding maintainer and restorer could offer further evidence for the thorough explaination.

References

Davis, W. H. 1986. Route to hybrid soybean production. United States Patent. US 4:545 146. and Biotechnology Advances 4(2):374-375.

Gai, Junyi et al. 1975. A preliminary study on the pollen viability of summer-planting soybean. Science and Technology of Oil Crops, (3):62- 67. (Chinese).

Peng, Yuhua et al. 1994. Genetic analysis of a new type of male sterile soybean. Abstracts of Papers WSRC V. p. 90. Funny Publishing Limited Partnership, Bangkok, Thailand.

Sun, Huan et al. 1994. A cytoplasmic-nuclear male sterile soybean line from interspecific crosses between Glycine max and Glycine soja. Abstracts of Papers WSRC V. p. 45. Funny Publishing Limited Partnership, Bangkok, Thailand.

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Screening of soybean germplasm for resistance to Sclerotinia sclerotiorum

With the increase of continuous soybean and soybean-corn tillage system (traditionally, soybean-corn-corn or soybean-corn-wheat), Sclerotinia stem rot of soybean, caused by Sclerotinia sclerotiorum, is becoming more and more serious in Heilongjiang Province of China. Soybean acreage used to be under 33% of the total planting acreage annually. It was effective in controlling some soil-borne diseases. But in recent years, soybean is more profitable as a cash crop than other field crops. Some farmers use more than 50%, even 60%, of their land to grow soybean. Sclerotinia stem rot occurs more frequently. To solve this problem, there are three choices: (1) to control soybean acreage under 33%; (2) to use chemicals; (3) to grow resistant cultivars. Growing resistant cultivars should be the best choice. Currently, there are no cultivars resistant to Sclerotinia sclerotiorum. The objective of this experiment is to screen soybean germplasm for resistance to Sclerotinia sclerotiorum.

Materials and Methods

Sclerotia were collected from diseased plants in the fields. Three hundred and fifty-nine germplasm lines were planted in May 4, 1991, in plastic pots with a diameter of 15cm, three seeds per pot, two replicates. Two pieces of sclerotia were put into each pot. The same method was used in 1992. Four hundred and fifty-five germplasm lines were planted in May 13, 1992, including 25 lines showing resistance in 1991. Plants were rated for disease severity according to a scale of 0-2, where 0 = no symptoms, 1 = lesions on the plant, and 2 = dead plant with sclerotia.

Results and discussion

Most lines screened in both years were highly susceptible to Sclerotinia

sclerotiorum (Table 1). Twenty-five lines showing resistance in 1991 were investigated in 1992, all were susceptible but one. Therefore, we cannot say that the 11 lines showing resistance in 1992 were really resistant. Most lines showing resistance were in the edges of the nursery, moisture may be the reason why these lines were not infected. The method used in this experiment is effective in discarding susceptible lines.

Table 1. Number and percentage of lines with different disease severity classes

		Disease Severity Classes[a]				
Year		0	0.5	1.0	1.5	2.0
1991	No. of Lines	25	16	96	85	137
	%	6.96	4.46	26.74	23.68	38.16
1992	No. of Lines	11	12	172	84	151
	%	2.56	2.79	40.00	19.53	35.12

[a]Means of two replicates.

References

Boland, G.J. and R. Hall. 1987. Evaluating soybean cultivars for resistance to Sclerotinia sclerotiorum under field conditions. Plant Disease 71:934-936.

Grau, C.R., V.L. Radke and F.L. Gillespie. 1982. resistance of soybean cultivars to Sclerotinia sclerotiorum. Plant Disease 66:506-508.

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Top Necrosis Symptom of the Soybean Plants Infected. by Soybean Mosaic Virus (SMV)

Top necrosis of soybean plants effected by SMV can cause serious harm. Kendrick and Gardner reported firstly this symptom in 1924. Epidemic of top necrosis disease ruined soybeans of several million hectares in southern Korea in 1974. Kwon found that the necrotic reaction in Kwanggyo was controlled by a recessive gene in 1980. Buzzell and Tu identified a dominant gene conditioning a necrotic reaction in Columbia in 1989. Bowers proved that resistance in HLS to SMV strain G_7 was conditioned by a single dominant gene in 1992. Recently, top necrotic disease is occurring more wide spread and menacing the selection and popularization of soybean vareties in China. The purposes of this study were to further investigate the phenotypes and inheritance of necrotic reaction and to control its occurrence and epidemic.

Materials and Methods

We choose resistant parents Xudou 2 and Liao 81-5017 from which the progenies appeared top necrotic plants and susceptible Jilin 19 and Suinong 4, and made 4 crosses in 1988. 4 back crosses were made and F_0 seeds derived from these crosses were planted separately in 1989. The parents, F_1, BCF_1 plants and F_2 populations from each cross were planted in Experimental field of Soybean Institute of Agriculture Academy, GongZhuling, with 60 m maze around on 15 May 1991. The bestards from F_1 plants were eliminated on the basis of leaf type and the colour of plumular axis after seedling. All plants were artificially inoculated by rubbing the blades at the first trifoliolate leaf stage. Inoculum was prepared by grinding leaves infected SMV strain Dongber 11 in 0.02 M sodium phosphate buffer at pH 7, with a mortar and pestle. Disease ratings were evaluated 4 weeks (fully show their symptoms) after inoculation. Plant resistance was classified 6 grades, 1-R, 2-MR, 3-

MS, 4-S and 5.6-HS, less and equal 2 grade were resistance and more 2 were susceptible.

Results

1. Necrotic reaction

Growing point and new leaves of effected plants firstly occurred brown necrotic spots, the number of spots increased, the size developed, and then the colour of vein changed brown, the petiole and stem were necrotic. Lastly growing point withered, the plants changed stunting and brittle and produced system necrosis after being inoculated with SMV strain Dongbei II, this process needed about 30 to 45 days.

The resistant parents Xudou 2 and Liao 81-5017 showed symptomless and light mosaic leaf and the susceptible Jilin 19 and Suinong 4 were severe mosaic wrinkle and so on after being inoculated. There were no top necrotic plants in these effected parents but there were many ones in F_1 progeny and F_2 population from their crosses (Table 1).

Table 1. Segregation of the parents, F_1 and F_2 plants from resistant parents crossed with susceptible ones to SMV II.

Crosses	Parents								Generations							
	P_1				P_2				F_1				F_2			
	T	R	S	TN	T	R	S	TN	T	R	S	TN	T	R	S	TN
Jilin 19 x Xudou 2	48	0	48	0	49	49	0	0	50	31	2	17	252	113	73	66
Jil.19 x L.81-5107	48	0	48	0	53	53	0	0	42	20	1	21	196	82	59	55
Suin. 4 x Xudou 2	54	0	54	0	49	49	0	0	51	15	1	35	249	105	69	75
Suin. 4 x L.81-6107	64	0	54	0	53	53	0	0	32	14	0	18	271	108	66	107

T: Total plants R: Resistant plants
S: Susceptible plants TN: Top necrotic plants

2. Top necrotic plants F_1 generation and F_2

F_1 plants of each cross segregated top necrotic plants from 1/3 to 2/3, the rest

were symptomless plants. There were three symptom types: symptomless, symptom (mosaic and wrinkle) and necrotic plants in F_2 population (Table 1). Bowers considered that necrosis was termed a hypersensitive form of resistance, and necrotic plants were included in the resistant class whenever genetic ratios were discussed. But Hong et al., considered necrosis was one of SMV symptoms and its plants ought to belong to susceptible class. We made inheritence analysis from two aspect. If top necrosis was resistant reaction, the population of F_2 plants from each cross would expect 3 resistance : I susceptible. Chi-square value had a probability >0.05. It showed the resistance was conditioned by a single dominant gene. If top necrosis was susceptible, all the F_2 progenies of these crosses would segregate in 7 resistant : 9 susceptible. Chi-square analysis showed that the resistance is under two recessive complementary genes controlling (Table 2) .

Table 2. Segregation of resistant of F_2 plants from resistant parents crossed with susceptible ones

Crosses	T	R	S	Exp. R.	χ^2	P
Jilin 19 x Xudou 2	252	113	139	7:9	0.122	0.76 - 0.5
	252	179	73	3:1	2.12	0.26 - 0.1
Jilin 19 x L.81-5017	196	82	114	7:9	0.292	0.76 - 0.5
	196	137	59	3:1	2.721	0.1 - 0.05
Suin. 4 x Xudou 2	249	105	144	7:9	0.253	0.75 - 0.5
	249	180	69	3:1	0.976	0.5 - 0.25
Suin. 4 x L.81-5017	271	108	163	7:9	1.684	0.25 - 0.1
	271	215	56	3:1	2.717	0.1 - 0.05

3. The top necrotic plants of backcrosses BCF_1 progenies

The plants of backcrosses BCF_1 progenies inocculated SMV mainly appeared two symptom types: resistant and top necrotic besides a few plants displaying wrinkle and dwarfing symptomes (Table 3).

If the top necrotic plants were thunk of resistant reaction the population of four crosses would not segregate in 1 resistant to 1 susceptible and appear all resistant plants. Conversely, of susceptible reaction they would be 3 resistant to 1 susceptible,

Chi-Square test had a goodness fit.

Table 3. Reaction of the back cross progenies of Jilin I9 and Suinong 4 to SMV II

Crosses	T	R	S	TN	Exp. R.	χ^2	p
(Jil. 19 x Xud. 2) x Jil. 19	55	41	1	13	3:1	0.006	> 0.9
(Jil. 19 x L.81-5017) x Jil. 19	30	22	0	8	3:1	0.042	0.9 - 0.75
(Suin.4 x Xud. 2) x Suin. 4	36	26	3	7	3:1	0.148	0.75 - 0.5
(Suin. 4 x L.81-5017) x Suin. 4	23	18	0	5	3:1	0.130	0.75 - 0.5

Discussion

Characters of monogenic control do not segregate in the F_1 plants of a cross. However, the F_1, plants could segregate symptomless plants and top necrotic plants in our test. This result was supported by many reports. Koshimizu and Iizuka explained that heterozygous plants can show necrosis, which may indicate incomplete dominance. Bowers found necrosis strongly associated with the heterozygous condition. The reason why not all heterozygous plants were necrotic was not known. Kiihl and Hartwing proposed that necrosis was a response to a heavy dosage of inoculum. Hovever, we will further study the inheritance phenomenon to get the true answer.

It is quite dispute that top necrotic reaction is conditioned by a dominant gene or by a recessive gene. We think that it is the key the top necrotic being resistant reaction or susceptible. Bowers considered that top necrosis was a resistant reaction and drew the conclusion that the resistance in HLS to SMV strain G_7 was conditioned by a single dominant gene. Kwon thought the top necrotic belonged to susceptible, its resistance was conferred by a result single recessive gene. Our study indicated, if the top necrotic was as resistant reaction its resistance would be underone dominant gene controlling, as susceptible its resistance would be two resessive complementary gene controlling. How evaluated the top necrotic reaction will be? As is well known, the purpose of inheritance study is serving the breeder in the crop breeding and the aim of the breeder is breeding high yield and resistant disease varieties. The top necrotic plants got few seeds and appeared not only in early generation and stable generation but also in varieties. We think it is incorrect that the top necrotic is as resistant reaction.

References

Kendrick, J.R., et al. 1924. Soybean mosaic: Seed transmission and effect on yield. J. Agric. Res. 27: 91-98.

Kwon, S.H., et al. 1980. Resistance to a necrotic strain of soybean mosaic virus in soybean. Crop Sci. 20:403-404.

Hong, E.H. 1987. Soybean varietal improvement and cultural practices in Korea. Crop Exp. Stn. Rural Devel. Administration. 30-33.

Bowers, G.R., et al. 1992. Inheritance of resistance to soybean mosaic virus in Buffalo and HLS soybean. Crop Sci. 32:67-72.

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Effects of leaf shape on seed yield and its components in soybeans

An obvious feature of soybean cultivars in southern China was less number of seeds per pod (NSPP). Soybean breeders believed that NSPP was related with leaflet shape, in other words, narrow leaflet type usually provided more NSPP. A greater number of cultivars in Northeast China and Huang-Hui-Hai Valleys were narrow leaflet type. But, so far, narrow leaflet trait has not been recognized and emphasized in the improvement of soybean yield in southern China. This paper deals with the effects and significance of leaf shape in the improvement of seed yield and NSPP in Nanjing, China.

Materials and Methods

Experiment 1

Three pairs of near isogenic lines (NIL) of leaflet shapes with different genetic background were developed by segregating from offspring of artificial or natural hybrids (Table 1). A factorial experiment was arranged in a split-split plot design with three pairs of NIL's in main-plot, two kinds of leaflet shapes (broad and narrow) in subplot and three planting densities (18.7, 25.0 and 31.2 plants/m^2) in sub-subplot factors, three replications, each plot 5 rows, 4 m long and 0.5 m apart. Growth analysis was conducted during growing season. At maturity, 10 plants from each plot were sampled to determine the yield components, and the central three rows of each plot were harvested for yield measurement.

Experiment 2

Seventy-two soybean cultivars (lines), three from the Northeast China, 22 from Huang-Hui-Hai valleys, 42 from southern China, 5 from abroad (U.S.A. and Japan), with broad and narrow leaflet were tested to further evaluate the effect of leaflet shape on seed yield and its components in a blocks in replication design with two replications, 6 sets, 12 cultivars in each set in 1992. And in 1993, 45 selected cultivars with relatively high

yield were further tested in a completely randomized block design with three replications. Each plot consisted of 5 rows, 4 m in length and 0.5 m apart in the two experiments. The procedures of measurement of yield and its components were the same as in Experiment 1.

Experiment 1 and Experiment 2 were conducted in Jiangpu Experimental Station of Nanjing Agricultural University. In addition, the leaflet shape of 729 cultivars, among them 341 cultivars with NSPP data, released from 1978 to 1982 in China in Hu and Tian (1993) were counted to analyze the geographic distribution and its relationship with NSPP.

Table 1. Performance and pedigree of three pairs of near isogenic lines

Near isogenic line	Leaflet shape	Flower color	Pubescence color	Plant height (cm)	Node number on main stem	Days to maturity (days)	Homogeneity	Pedigree
NIL90-4	Broad	White	Gray	64.7	13.8	112	93.75	Segregating from (Suxie 18-6 N6582)F4:5 line
	Narrow	White	Gray	56.5	13.8	114		
NIL91-1	Broad	White	Gray	78.2	20.7	117	93.75	Segregating from [(Nannong 493-1 x SRF400) x Nannong 493-1] F4:5 line
	Narrow	White	Gray	74.5	20.5	117		
NIL91-2	Broad	White	Gray	85.6	17.1	117	100	Segregating from a natural hybrid of N7623 plant
	Narrow	White	Gray	74.8	17.5	117		

Results and Discussion

(1) Between the broad and narrow leaflet types of the NIL's, there was no significant difference found in average seed yield and number of pods per plant, but it was found in 100-seed weight and number of seeds per pod (NSPP) (Table 2). There existed significant interaction between leaflet shape and planting density. The average yield of the broad leaflet NIL's from low to high density was 2503.5, 2457.0, 2476.5 kg/ha, respectively, and that of the narrow leaflet NIL's, 2304.0, 2484.0 and 2560.6

kg/ha respectively, which showed that the narrow leaflet type was favorable to yield at high density. At the same time, the narrow leaflet isolines produced more NSPP significantly than the broad ones did, in other words, the former produced much more 3 -seeded and 4-seeded pods per plant than the latter did. Leaf area indices (LAI) of the broad leaflet type of the two pairs of NIL's (NIL91-1 and NIL91-2) appeared to be significantly larger than the respective narrow leaflet type. But the specific leaf weight (SLW) of all the narrow leaflet NIL's was significantly larger than their respective broad leaflet ones (Table 3). A similar yield between two types of leaflet shape NIL's may result from the compensation between NSPP and 100-seed weight and between LAI and SLW.

Table 2. Seed yield and its components of three pairs of near isogenic lines

NIL	Leaflet shape	Yield (kg/ha)	NPP	NOSP	NTSP	NTHSP	NFSP	TNSP	RNSP	SW
NIL90-4	Broad	2395.5	51.8	3.1	42.6	6.1	0.0	2.08	1.56	15.4
	Narrow	2313.0	38.6	1.4	18.2	18.6	0.4	2.25	1.68	18.6
NIL91-1	Broad	2604.0	45.0	2.8	39.6	2.6	0.0	1.99	1.66	18.0
	Narrow	2562.0	41.2	1.2	17.5	22.4	0.1	2.48	2.02	15.9
NIL91-2	Broad	2437.5	38.8	1.4	26.8	10.6	0.0	2.21	1.60	18.3
	Narrow	2479.5	49.0	0.5	12.8	25.7	1.0	2.78	1.88	14.8
Average	Broad	2479.5	45.2	2.4	36.3	6.4	0.0	2.09	1.61	17.2
	Narrow	2451.0	39.9	1.0	16.2	22.2	0.5	2.50	1.86	16.4
Significance	NS	NS	**	**	**	**	**	**	**	

Note: NPP = number of pods per plant
NOSP = number of one-seeded pods per plant
NTSP = number of two-seeded pods per plant
NTHSP = number of three-seeded pods per plant
NFSP = number of four-seeded pods per plant
TNSP = theoretical number of seeds per pod
RNSP = real number of seeds per pod
SW = 100-seed weight.
* and **, significant at 0.05 and 0.01 probability levels, respectively.
NS = not significant.

(2) Seventy-two cultivars (1992) or forty-five cultivars (1993) from Northeast China, Huang-Huai-Hai valleys, southern China, U.S.A. and Japan, were divided into two groups of leaflet type, broad one and narrow one. The results confirmed that significant differences existed in NSPP, but not in seed yield and number of pods per plant (Table 4).

Table 3. Leaf area index (LAI) and specific leaf weight (SLW) of three pairs of near isogenic lines.

NIL	Leaflet shape	LAI			SLW (g/cm^2)		
		V_7	R_2	R_4	V_7	R_2	R_4
NIL90-4	Broad	0.89	3.17	3.12	0.36	0.31	0.44
	Narrow	1.05	3.30	3.68	0.42	0.32	0.40
NIL91-1	Broad	1.12	3.81	4.86	0.35	0.29	0.35
	Narrow	0.91	2.79	4.65	0.37	0.32	0.41
NIL91-2	Broad	1.00	3.62	4.50	0.43	0.32	0.44
	Narrow	0.96	3.09	4.28	0.45	0.33	0.44
Average	Broad	1.00	3.54	4.16	0.38	0.31	0.41
	Narrow	0.97	3.06	4.21	0.42	0.32	0.42
Significance		NS	**	NS	*	**	NS

Table 4. Seed yield and its components of cultivars with different leaflet shapes

Year	Leaflet shape	No. of cultivars	Yield (kg/ha)	No. pod /plant	100-seed weight	No. seeds per pod
1992	Broad	48	1638.0 ± 330.0	42.1 ± 9.3	17.5 ± 4.5	1.66 ± 0.34
	Narrow	19	1620.0 ± 396.0	42.5 ± 8.0	15.9 ± 2.6	1.90 ± 0.30
Significance			NS	NS	NS	**
1993	Broad	30	2310.0 ± 315.0	48.3 ± 13.3	20.7 ± 4.2	1.76 ± 0.23
	Narrow	12	2154.0 ± 280.5	39.6 ± 9.9	20.1 ± 3.0	2.07 ± 0.27
Significance			NS	NS	NS	**

(3) The results from 729 cultivars cultivated in China from 1978 to 1992 (Table 5) showed that the narrow (or lanceolate) leaflet type accounted for 45.4% cultivars in Northeast China, especially in Helongjiang and Jilin (71%) which produced more NSPP,

but in southern China, large parts of cultivars were of broad (ovate and elliptical) leaflet shape with less NSPP. In average, over all cultivars with NSPP data from different regions, the NSPP of narrow leaflet cultivars was higher than that of elliptical and ovate ones, being 2.15, 1.80 and 1.86, respectively.

Table 5. Geographical distribution of Leaflet shape and number of seeds per pod in soybean cultivars of China (1978-1992)

	No. cultivars/Percent					No. seeds per pod /No. cultivars				
Area	Round	Ovate	Elliptical	Narrow	Total	Round	Ovate	Elliptical	Narrow	Total
Northeast China	25/11.0	41/18.1	58/25.6	103/45.4	227/100	-	1.87/9	1.97/27	2.11/60	2.05/96
Huang-Huai-Hai Valleys	7/3.6	91/46.2	80/40.6	19/9.7	197/100	-	1.94/17	1.98/15	2.86/3	2.06/36
Southern China	6/2.0	135/44.2	158/51.8	6/2.0	305/100	-	1.85/127	1.73/105	2.02/3	1.79/209
Total	38/5.2	268/36.8	296/40.6	127/17.4	729/100	-	1.86/127	1.80/147	2.15/67	1.89/341

The above results demonstrated that gene resources with narrow leaflets may be used to improve the NSPP and canopy architecture of southern cultivars to enhance the NSPP and finally seed yield in soybean breeding programs in southern China.

References

Hartwig, E.E and C.J. Edwards, Jr. 1970. Effects of morphological characters upon seed yield in soybeans Agron. J. 62:64-65.

Hu, Mingxiang and Tian, Peizhan, 1993. Annals of Soybean Cultivars in China (1978-1992). Agriculture Press. Beijing.

Mandl, F.A. and G.R. Buss, 1981. Comparison of narrow and broad leaflet isolines of soybean. Crop Sci. 21:25-27.

Tian, Peizhan, 1977. Problem on leaf shapes in soybean breeding. Acta Genetica Sinica 4(1):22-30.

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Performance and inheritance of short petiole trait of a new soybean mutant

The enhancement of optimum leaf area index of a soybean population for better light-interception and photosynthesis may be realized by the aid of the development of cultivars favorable in narrow row-spacing. The short petiole trait may have value in altering the soybean canopy profile. Kilen (1983) and Tian et al., (1988) indicated that the inheritance of the short petiole trait was controlled by a single recessive gene. The present paper will report the performance and inheritance of the short-petiole trait of a new soybean mutant.

Materials and Methods

About 200 plants with distinctive short petioles were found in 1993 from the breeding line NJ90L-1 which had long inflorescence and normal petioles and was selected from the cross Suxie18-6 and N6582. Because all the plants and their progenies were completely uniform in the performance of short petioles and other traits in 1993 and 1994, it was inferred that the trait arose from a mutant and all the plants descended from it. The mutant plant may have been present in NJ90L-1 but not observed until 1993. Thus all the plants were harvested and designated as the short petiole parent, NJ90L-1sp.

Three single crosses plus their reciprocal crosses were made between NJ90L-1sp and Nannong 86-4, NJ90L-2 and D76-1609 in 1993 (Table 1 and 2). Both Nannong 86-4 and NJ90L-2 have long petioles and the latter is a sib-line of NJ90L-1 with long inflorescence and narrow leaflet. D76-1609 is a short petiole line selected by Kilen (1983). F_1 plants from the crosses were grown in the field of Hainan Island in winter of 1993. F_1 plants were harvested individually and two of the six crosses were again planted to obtain F_3 families. All the parents, F_1 plants, F_2 populations and F_3 families were grown in the field of the Jiangpu Experimental Station of Nanjing Agricultural University in 1994. The observation and measurement of the petiole traits of the third

leaf from the top were taken at the R_5 and R_7 stages.

Results and Discussion

(1) It was found that the short-petiole trait in NJ90L-1sp was completly linked with the abnomal pulvinus at the base of petioles. There was no enlarged pulvinus found in NJ90L-1sp, but in D76-1609 it was enlarged and normal.

(2) The results from the F_1 plants of six crosses (including the reciprocial crosses) showed that the short petiole trait with abnomal pulvinus was recessive to long petioles with normal pulvinus (Table 1 and 2).

Table. 1 The petiole length of the third leaf from the top in the six crosses

Cross	Generation	n	X	SE
	P_1=NJ90L-1sp	40	10.25	3.74
	P_2=Nannong 86-4	21	25.14	3.82
	P_3=NJ90L-2	19	22.53	2.89
	P_4=D76-1609	33	12.12	2.29
P_1 X P_2	F_1	7	28.57	2.76
	F_2	110	21.85	9.67
P_2 X P_1	F_1	8	28.75	4.77
	F_2	332	21.33	9.96
P_1 X P_3	F_1	11	26.91	1.38
	F_2	56	21.14	4.57
P_3 X P_1	F_1	9	24.22	2.91
	F_2	149	19.93	5.25
P_1 X P_4	F_1	5	29.20	3.03
	F_2	302	22.89	3.03
P_4 X P_1	F_1	5	26.00	3.16
	F_2	294	22.24	5.84

(3) The parents of crosses NJ90L-1sp X D76-1909 and D76-1609 X NJ90L-1sp were both of short petiole trait, but the petioles of F_1 plants of their crosses proved to be long (Table 1 and 2). It was inferred that the genes controlling short-petiole trait in the two parents was not allelic, and the interaction between them might exist.

Table 2. The segregation of short petiole trait with abnomal pulvinus in the six crosses

Cross	Gener-ation	No.units tested	No.units L Segreg-ation		S	Segreg-ation ratio tested	χ^2	P
P_1 X P_2	P_1	40			40			
	P_2	21	21					
	F_1	7	7					
	F_2	180	139		41	3:1	0.36	0.55
P_2 X P_1	F_1	8	8					
	F_2	348	263		85	3:1	0.03	0.85
	F_3	73	22	33	18	1:2:1	1.11	0.57
P_1 X P_3	P_1	8			8			
	P_3	19	19					
	F_1	11	11					
	F_2	148	135		13	15:1	1.22	0.27
P_3 X P_1	F_1	9	9					
	F_2	84	81		3	15:1	0.62	0.43
P_1 X P_4	P_1	40			40			
	P4	18	18					
	F_1	5	5					
	F_2	324	295		29	15:1	3.59	0.06
P_4 X P_1	F_1	5	5					
	F_2	302	288		14	15:1	1.08	0.30
	F_3	116	43	67	6	7:8:1	2.80	0.25

Note: 1. The unit forF_2 is a plant, while forF_3 is a family.
2. L=long petiole with normal pulvinus; S=short petiole with abnormalpulvinus; Segre.=segregation.

(4) The results from the two F_2 populations, NJ90L-1sp X Nannong 86-4 and Nannong 86-4 X NJ90L-1sp, showed a 3 (long petiole with normal pulvinus, abbreviated

as L) : 1 (short petiole with abnomal pulvinus, S) phenotypic segregation ratio from both individual cross and pooled analysis of the data, indicating single recessive gene inheritance (Table 2 and 3). In the pooled analysis, the heterogeneity χ^2 value was not significant, showing the consistency between the reciprocal crosses. The data of F_3 families of cross Nannong 86-4 X NJ90L-1sp fitted a 1(L) : 2(L+S) : 1(S) genotypic segregation ratio. The heterogeneity χ^2 was also not significant in the pooled analysis. Thus, the difference of single recessive gene inheritance of short-petiole trait with abnomal pulvinus between NJ90L-1sp and Nannong 86-4 was demonstrated and confirmed.

(5) The results of four F_2 populations from crosses NJ90L-1sp X NJ90L-2, NJ90L-1sp X D76-1609 and their reciprocal crosses fitted a 15 (L): 1 (S) phenotypic segregation ratio from both individual cross and pooled analysis(Table 2 and 3) , and not significant heterogeneity χ^2 among crosses was found. The data of F_3 Families of cross D76-1609 X NJ90L-1sp in Table 3 fitted the 7(L):8(L+S): 1(S) genotypic ratio. Therefore, the short-petiole trait with abnormal pulvinus was controlled by two duplicate recessive genes.

(6) It was inferred that the short petiole plants in NJ90L-1sp were of the genotype $lps_1lps_1lps_2lps_2$. Nannong 86-4 had the genotype $lps_1lps_1Lps_2Lps_2$ or $Lps_1Lps_1lps_2lps_2$, and NJ90L-2 and D76-1609 had the genotype $Lps_1Lps_1Lps_2Lps_2$. Interaction between genes controlling short-petiole traits in two parents NJ90L-1sp and D76-1609 will be further studied.

Table 3 Testing of the heterogeneity of segregation ratio in F_2 populations

Code of crosses	No. of crosses	L	S	Total	Segre-gation ratio tested	Pooled		Heterogeneity	
						χ^2	P	χ^2	P
P_1 X P_2, P_2 X P_1	2	402	126	528	3:1	0.31	0.58	0.09	0.76
P_1 X P_3, P_3 X P_1	2	216	16	232	15:1	0.07	0.79	1.77	0.18
P_1 X P_4, P_4 X P_1	2	617	42	659	15:1	0.003	0.96	3.62	0.06
P_1 X P_3, P_3 X P_1, P_1 X P_4, P_4X P_1	4	833	58	891	15:1	0.06	0.80	5.40	0.14

References

Kilen, T.C. 1983. Inheritance of a short petiole trait in soybean. Crop Sci. 23:1208-1209.

Tian Peizhan, Wang Jian and Shun Zhiqiang. 1988. Inheritance of short petiole character in soybeans. Soybean Sci. (China) 7(4):341-343.

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Distribution of seed size, protein content, fatty content of wild and landrace soybeans

Soybeans are widely distributed in China, due to their origin. There was separate information about their collection and identification. The objective of this study is to give general knowledge about the distribution of seed size, protein content and fatty content of wild and landrace soybeans in China.

Results

1. Seed size

Areas with the largest 100-seed weight of wild soybean are Heilongjiang, Jilin, Liaoning, Henan, Shanxi, Ningxia, Jiangsu, Anhui, Sichuan and Xizang. Seed sizes range from 2.18 to 3.30 g. Areas with the lowest weight are Neimeng, Yunnan, Guizhou and Guangxi, weights ranging from 1.05 to 1.09 g. Standard deviations are positively correlated with means among provinces.

For landrace soybeans, seed weight is 13 grams higher than wild soybeans. The largest seed size (greater than 15 g) are in Northeast, Shanxi, Ningxia, Jiangsu, Zhejiang, Sichuan and Xizang. Henan and Anhui are provinces with the smallest seed size (less than 12 g). From Table 1, the number of entries with 100-seed weight greater than 10 g in wild soybean and greater than 30 g in landrace soybean vary with provinces, for wild soybean, more in Jilin, Henan and Jiangsu, for landrace soybean more in Jiangsu, Zhejiang and Sichuan provinces.

2. Protein content

Protein content of wild soybean among provinces range from 38.13 to 47.41%. Entries above 45% protein content can easily be found in Heilongjiang, Liaoning, Henan, Gansu, Anhui, Hubei, Zhejiang, Jiangxi and Guizhou. Entries lower than 42% are mainly distributed in Ningxia, Shandong, Fujian and Xizang. Mean protein contents are not parallel with latitudes.

Table 1. Seed size, protein concentration and fatty concentration of wild soybean and landrace soybean from different areas

	Wild soybean (soja)							Landrace soybean (max)					
	Seed size (g/100 sd)		Protein content		Oil content			Seed size (g/100 sd)		Protein content		Oil content	
Origin	n	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	n	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Heilongjiang	739	2.55	1.91	46.35	2.67	9.54	2.94	632	19.60	3.26	43.52	2.07	19.02	1.69
Jilin	899	2.43	2.25	43.92	3.20	9.57	3.04	736	16.30	3.69	42.21	2.53	19.23	1.74
Liaoning	1100	2.39	1.50	45.18	2.77	10.60	2.44	752	18.49	3.99	43.50	1.99	18.97	1.57
Neimeng	179	1.09	0.13	43.89	0.84	9.80	0.60	187	13.12	4.61	43.46	2.67	17.82	1.54
Ningxia	103	2.18	1.41	38.13	1.24	16.08	1.51	88	16.46	2.76	44.36	1.55	17.10	1.31
Hubei	447	1.48	0.31	44.70	2.57	10.64	0.83	411	13.76	4.40	44.73	1.47	17.26	0.92
Sanxi	1741	1.47	0.81	42.73	3.54	10.05	1.81	1318	15.06	4.78	40.54	2.14	17.78	1.61
Shandong	134	1.78	0.77	40.78	10.67	10.39	1.82	224	12.59	3.71	43.27	1.98	18.01	1.62
Henan	305	3.30	2.81	45.36	2.75	12.06	2.65	48	11.32	4.63	43.71	1.74	19.42	1.18
Shanxi	400	2.26	1.57	44.12	3.76	11.89	2.34	796	12.04	4.54	43.91	2.44	16.94	1.29
Gansu	90	1.33	0.48	45.90	4.11	9.48	1.27	250			44.99	2.88	17.53	1.21
Jiangsu	110	2.56	2.60	43.80	2.28	10.60	2.46	193	16.72	8.21	44.89	2.23	18.43	1.06
Anhui	117	2.42	1.31	47.41	4.95	9.51	1.90	149	11.41	3.25	45.27	2.63	19.26	1.40
Hubei	70	1.55	0.71	46.73	2.11	11.15	1.48	888	12.93	3.33	45.44	2.24	16.78	1.44
Sichuan	35	2.51	1.82					1486	15.26	2.87	46.63	2.08	17.42	1.33
Zhejiang	166	1.73	1.18	46.64	2.34	9.81	2.18	240	21.82	6.57	44.83	1.70	18.11	0.87
Fujian	370	1.51	0.70	41.56	2.07	7.82	1.32	162	13.37	3.15	44.21	2.16	18.95	1.18
Jiangxi	64	1.41	0.86	46.19	1.99	9.33	1.46	281	14.60	3.64	47.05	2.15	17.83	1.18
Hunan	56	1.45	0.53	44.41	5.71	9.20	1.54	307	13.99	2.93	46.43	1.84	17.91	1.72
Guizhou	86	1.05	0.19	45.03	1.48	8.11	0.85	1302	11.60	2.82	46.26	1.80	16.98	1.02
Guangdong	17	1.06	0.18	42.61	1.52	10.26	1.08	245			44.16	1.26	18.39	0.81
Guangxi	90	1 .05	0.39	42.19	3.72	10.67	2.24	254	13.56	3.38	45.80	1.58	17.69	1.23
Yunnan	25	1.50	0.14	42.47	2.54	11.99	0.57	325			45.17	1.77	17.11	1.16
Xizang	11	2.13	.021	41.80	1.91	12.50	0.83	11			45.13	2.11	17.22	1.43

*n: number of entries, x: mean, s: standard deviation

For landrace soybeans, protein content ranges from 40.54 to 47.05% and decreases from south to north, e.g. entries in Huanan area, content is higher than 44%, while in northern area lower than 40%. In northern areas, e.g. Northeast area, contents

are lower than wild soybean, however, in southern area, situation is otherwise, while in central China, both cases showed up. Soybean entries which are above 45% are concentrated in Anhui, Hubei, Sichuan, Jiangxi, Hunan, Guizhou, Yunnan, Guanxi and Xizang. Province with the highest mean protein content, 47.05%, is Jiangxi.

From Table 2, wild soybeans with protein content over 55% are mainly in the Northeast area, Jiangsu and Henan provinces. Landrace soybean with protein content above 50% is mainly in Hubei, Sichuan, Jiangxi and Guizhou provinces.

Table 2. Number of entries reaching certain levels

	Wild soybean			Landrace soybean		
	nsww	npw	now	nswl	npl	nol
Heilongjiang	2	47		2		
Jilin	30	225	3			
Liaoning	1	32		4		1
Neimeng						1
Sanxi			1	2		5
Shandong		1				1
Henan	9	8				
Shanxi	1	3				
Gansu		2				
Jiangsu	8	12		68	1	
Anhui		7			4	2
Hubei		4			11	
Sichuan					51	
Zhejiang		6		56		
Fujian					1	1
Jiangxi					21	
Hunan					4	
Guizhou					15	
Yunnan					2	

nsww: number of wild soybean entries with 100-seed weight over 10 g
npw: number of wild soybean entries with protein content over 55%
now: number of wild soybean entries with oil content over 20%
nswl: number of landrace soybean entries with 100-seed weight over 30 g
npl: number of landrace soybean entries with protein content over 50%
nol: number of landrace soybean entries with oil content over 23%

3. Fatty content

Fatty contents of wild soybeans range from 7.82 to 16.08%, areas with more than 11% are Ninxia, Henan, Shanxi, Hubei, Yunnan and Xizang. The highest area is Ninxia, 16.08%. Contents are not varied parallel with latitudes. Fatty contents of landrace soybean vary from 16.98 to 19.23%. In the north, fatty content is higher than in south. Contents of landrace entries are about 7% higher compared with wild soybean. Entries of wild soybean with fatty content over 19% are distributed in Heilongjian, Jilin, Henan and Anhui. Entries of landrace soybean over 23% are distributed in Liaoning and Sanxi Provinces.

References

China's Crop Science Society. 1979. Proceedings on Crop Germplasm. Vol. 9. Nanjing Agricultural University.

China's Crop Science Society. 1980. Proceedings on Crop Germplasm. Vol. 1. Vol. 2. Nanjing Agricultural University.

China's Crop Science Society. 1986. Proceedings on Crop Germplasm. Soybean. Nanjing Agricultural University.

Germplasm Institute. China's Academy Agricultural Sciences. 1990. Catalog of China's Soybean Wild Soybean. Agricultural Publishing House.

Germplasm Institute. China's Academy Agricultural Sciences. 1991. Catalog of China's Soybean Landrace. Agricultural Publishing House.

Oil Crop Research Institute. China's Academy Agricultural Science. 1982. Catalog of China's Soybean Landrace (continued). Agricultural Publishing House.

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Genetic variability of agronomic and quality characters of land-race soybean the Mid-Yangtze River Valley

There are large a number of soybean land-race entries originating from the Mid-Yangtze River Valley (MYRV) due to its geological and climatic complexity. To reveal the genetic variability of soybean germplasm from this area is of great importance for soybean plant breeding.

Materials and Methods

94 soybean land-race entries randomly selected from germplasm population of MYRV and 6 widely cultivated varieties from Lower-Yangtze River Valley (LYRV) were used to study genetic variability of soybeans from MYRV with three replications in Nanjing. Each replicate was 5 rows, 4 meter long and 0.5 meter space between rows. A 2.5 square meter section of each plot was harvested for yield. 10 plants from each plot were taken for character measurements and 10-plant mean used as the plot mean.

Results

Characteristics of germplasm from MYRV (GM) compared with cultivars LYRV (CL) were as follows:

1. Longer growing period
   Mean growing period of CM was 13 days longer than that of CL. This difference resulted mainly from days to flower, whereas days from flower to maturity were very similar.

2. Higher plants

   Plants of GM were of 15.7 cm higher. The increase was closely associated with the length of growing period.

3. Among yield components

   Among yield components, greater number of pods per plant with fewer number of seed per pod and smaller seed. Pod number per plant of GM increased by 6, while number of seed per pod decreased by .3, seed size decreased by 4.3 gram.

4. Higher protein content and lower oil content

   Mean protein content increased by 2.2 %, while oil content decreased by 4.4 %. From data of ranges and genetic coefficients of variation (GCV), it could be seen that:

   a. GCV of growing period was small, the earliest variety taken 113 days to maturity, which is very similar to CL, therefore, it's unlikely to select entries with short growing period.

   b. GCV of such yield related characters as number of pods per plant, 100-seed weight, number of seed, seed weight per plant and yield were 21.7 25.6, 27.6, 26.1, and 28.0, gains of selection (GS) were 25.8, 49.3, 38.4, 24.3, 47.7 respectively. Selection in the population would be effective.

   c. Although GCV of protein content and oil content were small, entries with high protein content (> 45%) could easily be found in the area.

Table. Parameters of land-race soybean from MYRV and cultivated soybean from LYRV

	Mean		Range	GCV (%)	Gain of Selection
Character	MYRV	LYRV	MYRV	MYRV	MYRV
Days to flower	59.9	47.2	49.3-77.7	9.3	18.4
Days from flower to maturity	66.3	65.7	56.7-86.0	6.3	9.9
Days to maturity	126.2	112.9	113-146	4.7	8.6
Height (cm)	66	50.3	35.4-130.8	28.5	49.7
No. branch	3.2	3.4	1.2-5.9	24.9	33.4
Pod/plant	34.2	28.1	14.6-63.0	21.7	25.8

Table. continued

Character	Mean		Range	GCV (%)	Gain of Selection
	MYRV	LYRV	MYRV	MYRV	MYRV
Seed/Pod	1.4	1.7	1.0-2.3	9.7	9.2
100 seed weight (g)	13.7	18.0	8.3-29.8	25.6	49.3
Seed/Plant	47.7	44.8	17.3-88.8	27.6	38.4
Seed weight/plant (g)	5.9	7.6	2.4-21.8	26.1	24.3
Yield (kilogram/ac)	1427.3	1588.5	447-2604.8	28.0	47.7
Harvest index (%)	42	47	25-56	12.5	15.3
Protein content (%)	43.5	41.3	38.1-47.5	3.8	6.7
Oil content (%)	18.0	22.5	16.1-19.8	3.7	6.2

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The zymogram pattern of the new type of SBTi-A_2 of seed protein and the crossing with other alleles

We found one new germplasm which trypsin inhibitor zymogram pattern is different from Ti^a, Ti^b, and Ti^c from cultivated soybeans (Glycine max) in China in 1991. During 1992 and 1994 we grew it in Jilin Academy of Agricultural Sciences (Gongzhuling, Jilin Province, China), in greenhouse when in winter and in field when in summer. It was planted six times in 3 years. In the meantime we planted it in Jilin Province in 1992, and planted it in Shandong Province in 1993. 8 shares of seeds were gathered and detected by electrophoresis. The result coincided to that in 1991 completely. Tthis showed that the new type (Ti^x) has stable heredity. We purified Ti^x from these seeds for biochemical experiment. Reciprocal crossing was done using this variety as parent with the varieties of Ti^a, Ti^b, Ti^c, titi (L81-4590). We reported the purification of Ti^x and the electrophoretic result of F_1 seeds.

Material and Methods

Seeds for the purification and crossing and the seeds of other genotypes were gathered in 1993. PAGE technique was used in the experiment. Purification of Ti^x, soybean powder was degreased by aceton, and then seperated by the column of DEAE-52.

Preparation of samples for electrophoresis: the new type of SBTi-A_2 is SP_1^a type. In order to detect whether the hybrid is true or false, the varieties of Ti^a, Ti^b, Ti^c, which is SP_1^b type were used. We took a slice for sample preparation from the crossbred seed were far away from the embryo. The seed can also be used for reproduction.

Results and Discussion

Only one band appears when the purified Ti^x is detected by electrophoresis (Fig. 1), and its Rf value is the same as that of the variety of the new type. When trypsin

is added, the band of Ti^x disappeared and a spot of compund of inhibitor - trypsin appeared above, this the same as the reaction of SBTi-A_2 standard with trypsin.

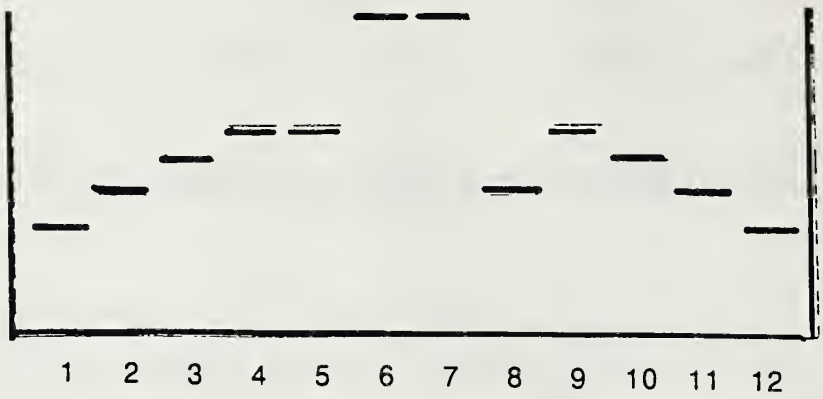

Fig. 1 Electrophoresis of purified Ti: 1, 12-Ti^c; 2, 11-Ti^a; 3, 10-Ti^b; 4, 9-Ti^x; 5-purified Ti^x; 6-Ti^x + trypsin; 7-standard + trypsin; 8-standard.

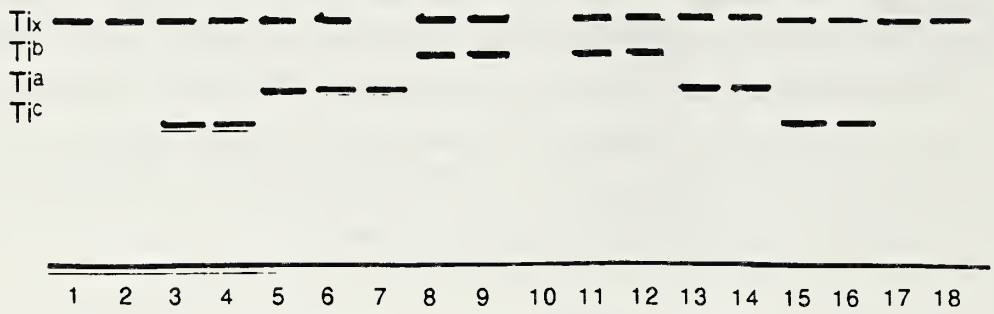

Fig. 2 The reciprocal crossing results of the alleles of SBTi-A_2 (Ti^a, Ti^b, Ti^c, titi) with Ti^x : 1, 2-titi x Ti^x; 3, 4-Ti^c x Ti^x; 5, 6-Ti^a x Ti^x; 8, 9-Ti^b x Ti^x; 11, 12-Ti^x x Ti^b; 13, 14-Ti^x x Ti^a; 15, 16-Ti^x x Ti^c; 17, 18-Ti^x x titi; 7-false hybrid (female Ti^a).

The alleles of SBTi-A_2 (Ti^a, Ti^b, Ti^c, titi) and Ti^x were crossed each other the zymogram pattern of seed protein of F_1 seed of reciprocal-crossing was the same (Fig. 2). This proved that the effect of reciprocal-crossing does not exist and the inheredity of Ti^x is controlled by the karyon gene.

Reference

Zhao Shuwen and Wa Hai. 1992. Soybean Genetics Newsletter, Vol. 19:22-24.

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Inheritance of a new variant of SBTi-A_2 in seed protein of soybean (Glycine max) in China

The Kunitz trypsin inhibitor was found in 1945 in soybean seed protein. It was designated SBTi-A_2 through electrophoresis and genetic study by Singh et al. (1969), Hymowitz, Orf, Kaizuma (1972, 1973, 1978, 1981). There were three dominant alleles, named Ti^a, Ti^b, Ti^c, and one recessive allele (titi) separately.

The author had found a new type of SBTi-A_2 for 11081 Chinese soybean (Glycine max) germplasms (1992, 1993).

Materials and Methods

F_2 seeds from the crosses of Ti^b x Ti^x, Ti^a x Ti^x, and titi x Ti^x varieties (titi-L81-4590, from U.S.A.), were analyzed for the electrophoretic band (PAGE).

Results and Discussion

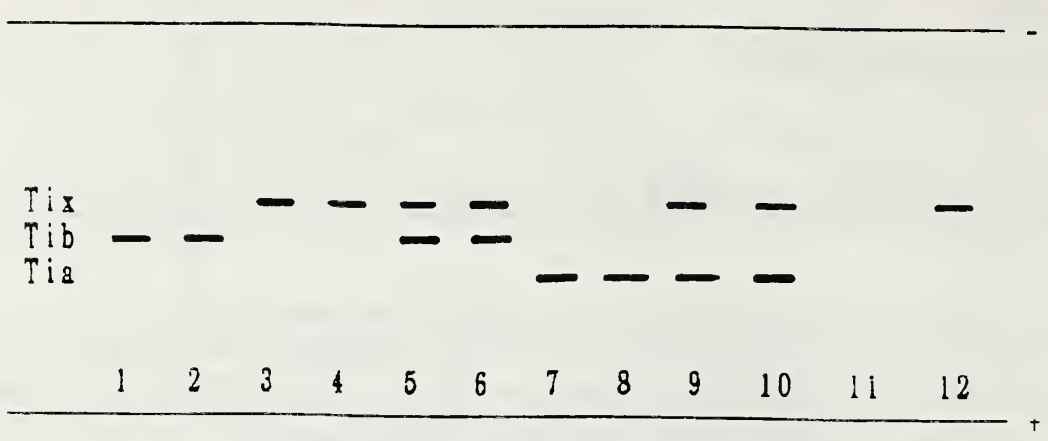
The F_2 seeds protein of Ti^b x Ti^x and Ti^a x Ti^x had two single bands with different Rf and a series of double-bands, but most of the ti x Ti^x F_2 seeds have a band, only a few of the seeds don't have bands.

1. Comparison of SBTi-A_2 Rf between electrophoretic band of F_2 seeds protein and electrophoretic bands of parent.

The Rf of the two single-bands were consistent with Ti^b (female) or Ti^a (female) and the new type (male), respectively, and the double-bands consistent with female and male, respectively, suggesting that the results were due to the inheritance of male/female.

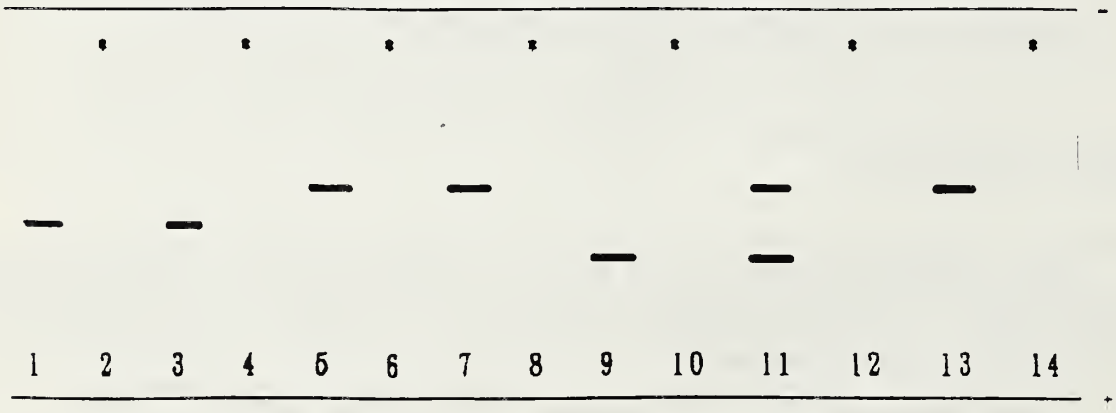
2. Reaction of F_2 seed protein sample to plus trypsin

Electrophoretic bands of seeds sample disappeared when adding trypsin (see Fig. 2, lanes 2, 4, 6, 8, 10, 12, and 14) same as the parents.



1. Ti^b female
2. Ti^b x Ti^x F_2 seeds fast-moving bands
3. SBTi-A_2 Ti^x (male) - seed of SBTi-A_2 new type
4. Ti^b x Ti^x F_2 seeds slow-moving bands
5. Ti^b/Ti^x samples of Ti^b (female) plus Ti^x (female)
6. Ti^b x Ti^x F_2 seeds double-bands
7. Ti^x (female)
8. Ti^a x Ti^x F_2 seeds fast-moving bands
9. Ti^a/Ti^x samples of Ti^a (female) plus Ti^x (female)
10. Ti^a x Ti^x F_2 seeds double-bands
11. titi (female) - Recessive genotype
12. ti^x Ti^x F_2 seeds

(The Rf of Ti^a is 0.85, Ti^b-0.82, a new type Ti^x is 0.79 in our laboratory conditions)



1. Ti^b female
2. Ti^b plus trypsin
3. Ti^b x Ti^x F_2 seeds fast-moving bands
4. Ti^b x Ti^x F_2 seeds fast-moving bands plus trypsin
5. Ti^x (male)
6. Ti^x plus trypsin
7. Ti^b x Ti^x F_2 seeds slow-moving bands
8. Ti^b x Ti^x F_2 seeds slow-moving bands plus trypsin
9. Ti^a (female)
10. Ti^a plus trypsin
11. Ti^a x Ti^x F_2 seeds double-bands
12. Ti^a x Ti^x F_2 seeds double-bands plus trypsin
13. ti x Ti^x F_2 seeds
14. ti x Ti^x F_2 seeds plus trypsin

* Compound of SBTi-A_2 and trypsin

3. χ^2 test of segregation proportion of electrophorectic bands. It could be considered that genotype of the new type of SBTiA_2 was different for Ti^a, Ti^b, and Ti^c. Perhaps this is a new allele. We are continuing inheritance research to confirm it.

Table 1. Observed and expected segregation of electrophoretic bands of SBTi-A_2 in F_2 seeds from the single plant of the crosses Ti^b x Ti^x (a new type) F_2 and Ti^a x Ti^x F_1 seeds

Crosses	No. of seeds		F_2 electrophoretic bands			χ^2	Probability
Ti^b x Ti^x	212	Rf	0.82	0.82/0.79	0.79		
			Ti^b	Ti^b/Ti^x	Ti^x		
		Observed	51	97	64	3.123	≅ 0.25
		Expected	53	106	53		
Ti^a x Ti^x	268	Rf	0.85	0.85/0.79	0.79		
			Ti^a	Ti^a/Ti^x	Ti^x	6.283	≅ 0.04
		Observed	60	154	54		
		Expected	67	134	67		

χ^2 test showed that segregation porportion of bands of SBTi-A_2 in the F_2 seeds were fitted to the 1:2:1 of Mendelian inheritance law.

Table 2. Observed and expected segregation of electrophoretic bands of SBTi-A_2 seeds from the single plant of the cross titi x Ti^x. F_1 seed

Cross	No. of Seeds		F_2 electrophoretic bands No SBTi-A2 Rf 0.79		χ^2	Probability
			titi	Ti^x		
titi x Ti^x	444	Observed	115	329	0.129	>0.5
		Expected	111	333		

χ^2 test showed that segregation porportion of bands of SBTi-A_2 in the seeds were fitted to the 3:1 of Mendelian inheritance law.

References

Hymowitz, T. and N. Kaizuma. 1978. Soybean Genet. Newsl. 5:19-24.

Hymowitz, T. and H.H. Hadley. 1972. Crop Sci. 197-198.

Kunitz, T. 1945. Science. 10:668-669.

Orf, J.H. and T. Hymowitz. 1977. Crop Sci. 17:811-813.

Singh, L. et al. 1969. Crop Sci. 9:489-491.

Zhao Shuwen and Wang Hai. 1992. Soybean Genet. Newsl. 19:22-24.

Zhao Shuwen et al. 1993. Soybean Genet. Newsl. 20:45-47.

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Pre- and post-flowering photoperiod responses in early soybean varieties

When early soybean varieties were treated with short days (SD), the days to flowering from emergence were not significantly delayed. Therefore, these varieties were considered to be insensitive to the photoperiod (Garner and Allard, 1920; Wang et al., 1956). Liu et al., (1983) and Xu et al., (1990) reported that the pre-flowering SD treatment hastened the post-flowering development, although SD did not significantly change the length to flowering from emergence. In order to study the effects of pre- and post-flowering photoperiods on the whole growth period of early soybeans and to compare the photoperiodic sensitivity before and after flowering in these varieties, 2 pot experiments were carried out in this study.

Materials and Methods

Experiment I.

This experiment was conducted in 1991 at Northeast Agricultural University, Harbin (45° 41' N). The following varieties were used: Dongnong 36 (MG 000), TXHD (Taixingheidou) (MG 0), Hefeng 25 (MG I), BLBYS (Bolibaiyesheng) (MG I), and Jilin 20 (MG II). Two photoperiod treatments were carried out before flowering: (1) Natural photoperiod (NPP) in Harbin (the average daylength to R1 from emergence was about 14.5h. The twilight was estimated as 1h (Polson,1972). (2) Short day (SD): 12h. After flowering (R_1), the plants of both treatments were moved to the natural photoperiod (the average daylength to R_8 from R_1 was about 14.2h). On May 14, for each variety in each treatment, two seeds were sown per pot, 5 pots or 10 plants. The dates of emergence and R stages (Fehr et al.,1977) were recorded for each 2 days during the life.

Experiment II.

This experiment was conducted in 1992. The varieties were the same as in

Experiment I. Dongnong 36 and Hefeng 25 were sown on May 22, others were sown on May 10. Two photoperiod treatments were carried out as follows: (1) NPP: Grown under natural photoperiod in Harbin during the life (the average daylength to frost from R_1 was about 14.2h). (2) NPP+SD: Grown under NPP before flowering. After R_1, the plants were moved to SD (12h). The developmental stages were recorded as Experiment I.

Results

I. The effects of pre-flowering photoperiod on the post-flowering development

The result in Table 1 showed that the SD did not greatly change the duration to the beginning of flowering from emergence (V_e-R_1) but hastened the post-flowering development of the varieties. The value of RAE (Rate of after-effect) was much higher than HRF (Hastening rate of flowering). It can be drawn that the early varieties were quite sensitive to photoperiod if the whole growth period was considered.

Table I. The effects of pre-flowering photoperiod on the post-flowering development of soybean varieties (Harbin, 1991) (days)

	V_e-R_1		R_1-R_3		R_3-R_5		R_5-R_7		R_1-R_7			
Varieties	NPP	SD + NPP	NPP	SD + NPP	NPP	SD + NPP	NPP	SD + NPP	NPP	SD + NPP	HRF (%)	RAE (%)
Dongnong 36	36.3	36.5	6.0	3.4	9.8	9.3	29.6	22.4	45.4	35.0	-0.6	22.9
Hefeng 25	36.3	34.9	19.0	5.4	21.6	13.8	40.1	30.9	80.7	50.1	3.9	37.9
BLBYS	40.1	36.0	30.4	5.7	14.0	12.4	31.9	27.1	76.3	45.2	10.2	40.8
Jilin 20	40.2	36.4	23.9	7.6	15.3	13.8	36.9	34.0	76.1	55.4	9.5	27.2
TXHD	46.8	39.1	23.9	7.9	9.8	12.7	31.8	25.8	65.1	46.4	16.5	28.7
$\bar{x}$	A 39.9	B 36.6	A 20.6	B 6.0	a 14.1	a 12.4	A 34.1	B 28.0	A 68.7	B 46.4	8.3	32.5

1. HRF (Hastening rate of flowering) (%)= [(Days to R_7 from R_1 under NPP)-(Days to R_7 from R_1 under SD)] x 100% /(Days to R_7 from R_1 under NPP).
2. RAE (Rate of after-effect) (%) = [(Days to R_7 from R_1 under NPP)-(Days to R_7 from R_1 under SD+NPP)] x 100% / (Days to R_7 from R_1 under NPP).
3. NPP: Grown under natural photoperiod in Harbin.
4. NPP+SD: Induced to R_1 with short days of 12h. After R_1, the plants were under natural photoperiod.
5. V_e-R_1: The days to R_1 from emergence in Harbin.

2. The response of soybean plants to the post-flowering photoperiod

The reproductive stages of early soybean varieties in Experiment II were significantly changed by the post-flowering photoperiod. The durations of R_1-R_5, R_5-R_7 and R_1-R_7 were greatly shortened by SD ($P<0.01$) (Table. 2). HRM (Hastening rate of maturation) was higher than HRF (Table 1). It was indicated that the post-flowering photoperiodic response of the early soybean varieties was more sensitive than that before flowering.

Table 2. The responses of non-SD-induced soybean plants to the post-flowering photoperiodic treatments (Harbin, 1992) (days)

	R_1-R_5		R_5-R_7		R_1-R_7		R_1-R8		
Varieties	NPP	NPP + SD	NPP	NPP + SD	NPP	NPP + SD	NPP	NPP + SD	HRM (%)
Dongnong 36	18.8	16.2	24.2	24.0	43.0	40.2	50.7	45.2	6.5
Hefeng 25	28.8	22.7	39.5	32.0	68.3	54.7	76.8	61.0	19.9
BLBYS	28.8	25.2	37.0	25.3	65.8	50.5	75.7	57.5	23.3
Jilin 20	32.0	26.7	41.8	34.2	73.8	60.8	R_7	71.0	17.6
TXHD	24.0	21.0	33.0	26.2	57.0	47.2	68.2	61.0	17.2
$\bar{x}$	A 26.5	B 22.4	A 35.1	B 28.3	A 61.6	B 50.7	A >67.9	B 59.1	16.9

1. NPP: Grown under natural photoperiod in Harbin.
2. NPP+SD: Grown under natural photoperiod before R_1. After R_1, the plants were treated with short days of 12h.
3. HRM (Hastening Rate of maturation)(%) = [(The length to R_7 from R_1 under NPP)-(The length to R_7 from R_1 under SD)] x 100% / (The length to R_7 from R_1 under NPP.

Discussion

Our results agreed with the former reports that the early soybean varieties were insensitive to photoperiod in flowering (Garner and Allard, 1920; Wang et al., 1956). However, it was found that the post-flowering development of these varieties could be affected both by pre- and post-flowering photoperiods. When the whole growth period was considered, the early varieties were quite sensitive to photoperiod, especially at the reproductive period.

References

Garner, W.W. and H.A. Allard. 1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J. Agric. Res. 18:553-606.

Han, T.F. 1994. Studies on the post-flowering photoperiodic responses in the different ecotypes of soybeans. Phd dissertation. Library of Northeast Agricultural University.

Liu, H.Z., H.X. Liang, and J.F. Zhang. 1983. The after-effect of light duration on the development of soybeans. Acta Agriculturae. Universitatis Pekinensis 9(3):67-72.

Polson D.E. 1972. Day-neutrality in soybeans. Crop Sci. 12:773-776.

Wang, J.L., Y.X. Wu, H.L. Wu, and S.C. Sun. 1956. Studies on photoperiodic response of soybean varieties originating from different localities of China. Acta Agricultura Sinica 7(2):169-180.

Xu, L.K., J.C. Zhong and H.Z. Liu. 1990. The after-effect of photoperiod on the development and phenological traits of soybeans. Chin. J. Agric. Meteorol. 11(l):22-25.

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Impact of several factors related to inoculum, explants, compound and growth medium on tumorigenesis in vitro culture of soybean (Glycine gracilis and G. max)

Soybean breeding is recently limited to a narrow base since traditional techniques restrict effective introgression of new characters from other species into soybean varieties. Therefore, soybean is an ideal crop for gene manipulation.

To date, Agrobacterium tumefaciens has been the most widely used vector for introducing T-DNA into dicotyledonous plants (Hooykaas, 1989). In contrast to other crops, most reports had concluded that A. tumefaciens were avirulent or weakly virulent on soybean (Michael et al., 1987), which was also confirmed in our previous work (unpublished). In order to enhance introducing efficiency of T-DNA into soybean genome, an optimal in vitro infection procedure of A. tumefaciens is absolutely available to be constructed.

With regard to inoculation, it has been known that the transfer of T-DNA is mediated by virulence genes, which form the vir region of the Ti plasmid, and chv genes, found on the bacterial chromosome. Transcription of the vir region is induced by various phenolic compounds, such as acetosyringone and alpha-hydroxy-acetosyringone (Stachel SE et al., 1985). Other experiments have demonstrated that effective vir induction requires a medium with pH<5.7 and a carbon source such as sucrose (Alt-Moerbe et al., 1988). Meanwhile, the density of Agrobacterium has been identified to affect the capacity of gene transfer in some crops (Melanie E. et al., 1991).

Additionally, soybean embryogenesis system has been well established in some laboratories and become a popular mediate to gene transformation (Wenbin Lee et al., 1990), and it seems to be necessary to determine the suitable stage of immature embryos and an optimal initial time of inoculation. Moreover, plant growth regulators have been employed in some plant culture media to promote tumorigenesis. Nevertheless, less information is available in soybean hitherto.

Materials and Methods

Explant preparation

Soybean accession Kou 502 (Mashokutou), Peking 501, Toyosuzu and Keburi were planted in the field of National Institute of Agrobiological Resources in Japan. Immature seeds 3-10 mm long were harvested and cut to two pairs of explants.

Bacterial strains

Two wild-type Agrobacterium tumefacien strains were used for co-cultivation. These were nopaline strain A208 and agropine strain A281. Stocks of all strains were maintained on YEB agar medium.

Co-cultivation

For co-cultivation using all strains, YEB culture were grown overnight at 28 C° with 160 rpm shaking. The cells were then adjusted to 5×10^1 ; 5×10^4 ; 5×10^6 ; 5×10^8 and 5×10^{10} CFU/ml. Cotyledonary explants were inoculated by keeping bacterial inoculum driped around wounded area.

Co-cultivation medium consisted of MS basal medium with 2% sucrose, 0.22% gelrite, 0-10mg/L NAA, 0-5 mg/L BA, and 0-20 mg/L 2,4-D, adjusted to one of five different pH levels (pH 5; 5.5; 5.8; 6.5; 7.0) with 1M NaOH, with or without one of five concentration of acetosyringone (O; 20; 40; 100; 300 µM). Co-cultivation continued for 48 hrs under controlled conditions.

Tumour culture

After co-cultivation, explants were transferred to the same medium, except adding 250 mg/L carbenicilin to kill the inoculum. Frequency of tumorigenesis was recorded as number of explants forming tumours after 30 days. Complete randomized design was adopted with two replicates.

Results and Discussion

Effect of bacterial concentration on transformation

Tumorigenesis was low at 5×10^1 to 5×10^4 CFU/ml, increased significantly to its maximum at 5×10^6 CFU/ml and decreased at higher concentrations 5×10^8 to 5×10^{10} CFU/ml. The result was uniform with the report on Solanum tuberosume

(Messaoud Boudjeniba et al., 1990). There was a curvilinear relationship between the percent transformation and bacterial concentration (p=0.01). When compared to strain A208, a lower percentage of cotyledons were transformed at all levels of strain A281 (Fig 1). Generally, the number of tumours per cotyledon ranged from one to three. Bacterial concentrations of 5 X 10^6 and 5 X 10^8 resulted in the largest tumors.

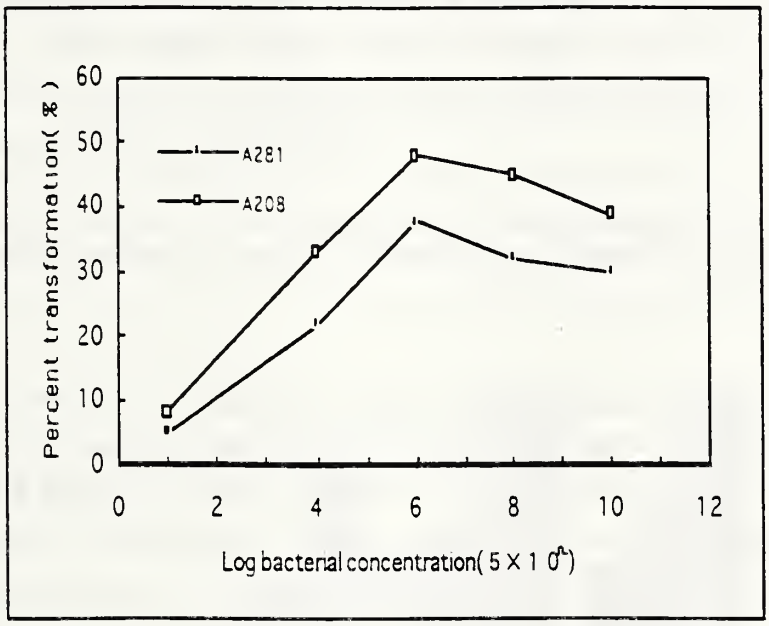

Fig 1. Effect of A. tumefacien strains A208 and A281 concentration on percent transformation of cotyledons derived from Kou5O2

Effect of pH and a phenolic compound acetosyringone on transformation

Presence of acetosyringone at co-cultivation greatly affected the virulence of both A208 and A281 in all pH's. Averagely, following increment of concentration of acetosyringone from 20 to 200 µM, the transformation was facilitated obviously. This was the most outstanding with acetosyringone 40 µM. However, higher concentration 300 u M depressed virulence of A.tumefaciens critically (Fig 2).

Additionally, results exhibited that pH was an important factor for vir-induction of A. tumefaciens. Transformation was promoted by lower pH, the optimal pH value was 5.8 pH. A pH higher than 5.8 reduced the tumorigenesis. Especially, pH 7.0 greatly inhibited tumorigenesis. There was a significant interaction between pH and acetosyringone. The treatment pH 5.8 with acetosyringone 40 µM contributed the most efficient transformation (Fig 2).

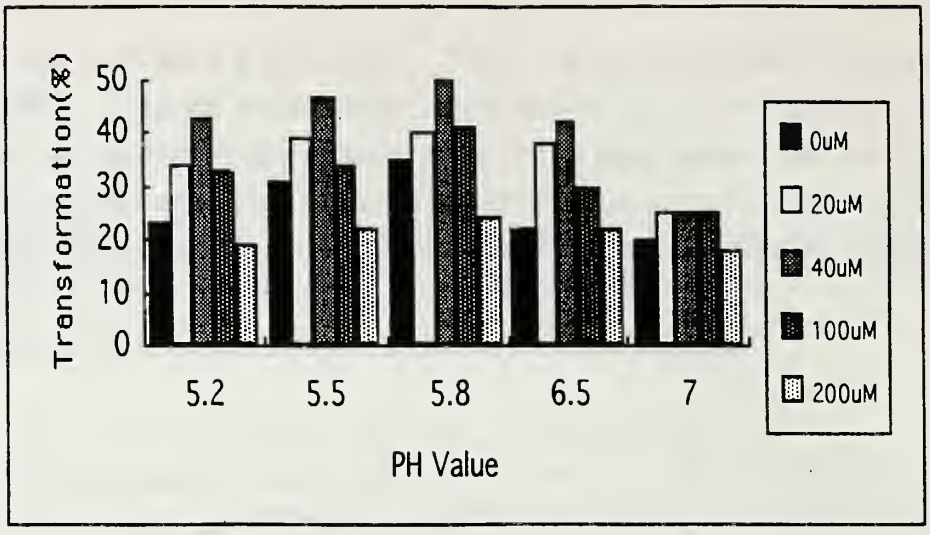

Fig 2. Number of explants forming tumors after co-cultivation with A208 and A281. The accession is Kou5O2 and the bars are represented as acetosyringone content.

Effect of initial time for co-cultivation on transformation

Cotyledons of Kou 502 were used to detect the optimal initial time for co-cultivation. In four time treatments, cotyledons, which were cultured for one day before inoculation with A. tumefaciens, possessed a higher percent transformation than inoculation with bacteria immediately, indicating that premitoses and DNA synthesis were required in host cell around wounding tissue for the incorporation of plasmid T-DNA. Nevertheless, long interval between preculture and co-cultivation led to a decrease of percent transformation (Fig 3).

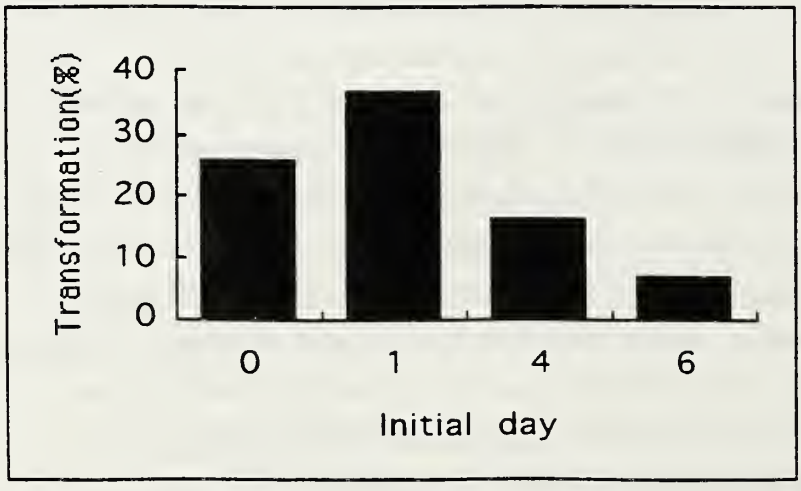

Fig 3. Effect of initial time of co-cultivation on percent transfer with A208 and A281 in accession Kou502

Effect of immature embryo stage on transformation

After inoculation with strains A208 and A281, cotyledons of all stages from 3 mm to l0 mm long formed tumors although there was a significant difference in percent tumorigenesis among different ages of cotyledons (P=0.01) (Fig 4). Older stage of cotyledons displayed a higher percent transformation than younger cotyledons in all three accessions. The moderate cotyledon stage was >6 mm long. Since cotyledons 3-5 mm long generally was the most competent to somatic embryogenesis (T. Komatsuda et al,. 1992), there seemed to be a barrier for successful transformation of expected genes into soybean by using younger immature cotyledon as explants according to some embryogenesis system.

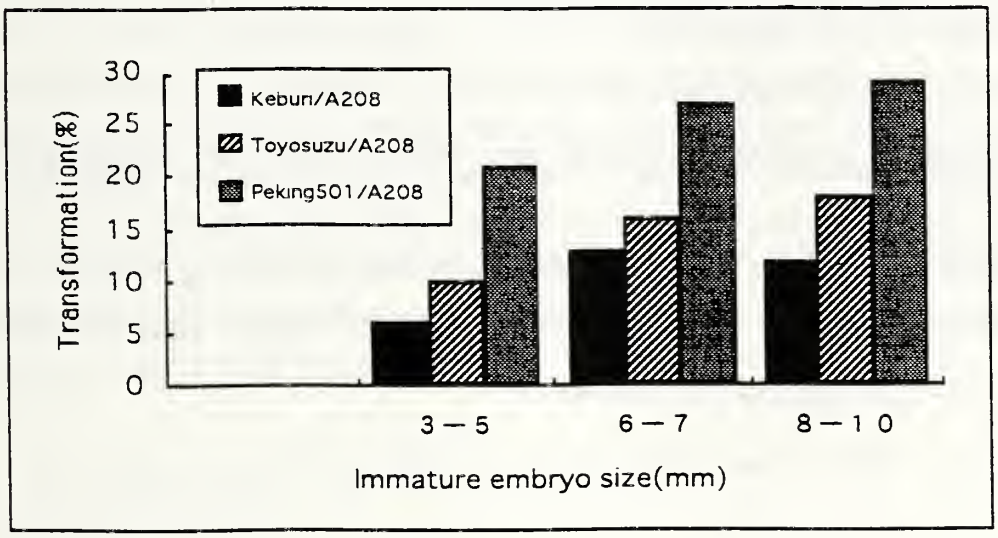

Fig 4. Effect of immature embryo stage on transformation

Effect of plant growth regulators on transformation

The addition of plant regulators NAA and 2,4-D to MS medium seriously depressed tumorigenesis (Table 1). NAA and 2,4-D resulted in a fifty-fold decrease of tumorigenesis in level 10 mg/L and 20 mg/L, respectively. Whilst, BA acted in an opposite way when it was added into culture medium. The effect of BA was additive to tumorigenesis, verifying that a plant regeneration system including BA as a source of phytohormone was necessary for an effective gene transformation in soybean.

Table 1. Effect of plant growth regulators on percent transformation

Regulators	Concentrations (mg/L)						
	0	0.1	0.5	1	5	10	20
	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$
NAA	38.4 ± 5.1			39.7 ± 7.5	26.0 ± 4.1	19.6 ± 4.1	
BA	38.4 ± 5.1	36 ± 6.6	41.4 ± 2.5	44.8 ± 4.3			
2,4-D	38.4 ± 5.1			36.5 ± 3.9	32.2 ± 6.2		21.7 ± 5.5

Reference

Alt-Moerbe et al. 1988. Mol Gen Genet 213:1-8.

Hooykaes P.J.J. 1989. Plant Mol Biol 13:327-336.

Messaoud Boudjeniba et al. 1990. Annales des sciences nalurelles, Botanque 13 (10):134-148.

Melanie E. Davis et al. 1991. Plant Cell,Tissue and Organ Culture 24:115-121.

S. Michael and C.Byme et al. 1987. Plant Cell, Tissue and Organ Culture 8:3-15.

Stachel SE et al. 1985. Nature 318:624-629.

T. Komatsuda and Wenbin Lee. 1992. Plant Cell, Tissue and Organ Culture 28:103-113.

Wenbin Lee and T. Komatsuda. 1990. Soybean Genetics Newsletter Vol. 17.

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Study on the oil content and fatty acid composition of soybean cultivars cultivated in Heilongjiang Province in China

Heilongjiang Province is one of the major soybean cultivation areas in China. Soybeans produced here are famous for their better quality and higher fat content. In recent years, research emphasis has been placed on nutritional quality such as protein content and fatty acid composition. Soybean oil contains some essential fatty acids, among of which, the linoleic acid can inhibit the increase of blood cholesterol. Thus, linoleic acid plays an important role in the prevention of high blood pressure and heart disease. On the other hand, the linolenic acid has been found to be the unstable component that is the determining factor of oil quality. Therefore, one soybean breeding objective is to increase the content of linoleic acid and to decrease the content of linolenic acid in order to improve the quality of soybean oil. For this purpose, the fat content and the fatty acid composition of soybeans cultivated in Heilongjiang Province has been determined in this paper.

Materials and Methods

70 soybean cultivars were collected from 60 counties located in Shonghuajiang, Suihua, Qiqihar, Jiamusi, Heihe, Mudanjiang and Harbin administrative districts in Heilongjiang Province. The oil content was determined with residue method and the fatty acid composition was analyzed by gas chromatography. The equipment was Hitachi Chromotographor Type 163. The results were reported by the microcomputer assembled on the equipment.

Results and discussion

1. Oil content of different soybean cultivars in Heilongjiang Province

The results obtained in the experiment showed that the mean oil content of 70 cultivars was 19.2%. and ranged from 17.6 to 23.4% (Table 1). Cultivar Nengfeng 10 had the highest oil content with 23.4% and cultivar Heihe 7 had the lowest with 17.6%. Among the 70 cultivars, 25 cultivars (36%) had an oil content of less than 20%, 40 (57%) had oil content ranging from 20.0 to 22.9%, and the remaining 5 (7%) had an oil content greater than 23.0% (Table 2). The 5 cultivars containing higher oil content were Nengfeng 10, Hongfeng 3, Heinong 31, Heinong 32, and Beifeng 2.

Table 1. Oil content of different soybean cultivars

Number of cultivars	Mean content (%)	Range of content (%)	Standard deviation	Coefficient variability (CV%)
70	19.2	17.6-23.4	1.2	6.1

Table 2. Range of oil content of soybean cultivars

Range of content	Number of cultivars	Percentage (%)
17.6-19.9%	25	36
20.0-22.9%	40	57
>23.0%	5	7

2. Fatty acid composition of oil different cultivars in Heilongjiang provice

The data studied on 70 soybean cultivars cultivated in Heilongjiang Province showed that the fatty acid of soybean oil mainly consisted of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. The content of linoleic acid ranged from a high of 53.99% among the 5 fatty acids (Table 3). This acid played a very important role in the better quality for Heilongjiang oil. On the other hand, the fact that the content of oleic acid was little lower than that of linolenic acid should be paid attention in the future breeding program.

3. The content variation of 5 fatty acids

Palmitic acid is a saturated fatty acid. The content of the acid varied in different

cultivars. The palmitic acid content ranged from 8.2 to 13.5%. The palmitic acid content of 58 cultivars (82%) ranged from 9.2 to 12.3%. The coefficient variability was 9.65%.

Table 3. Fatty acid composition of 70 soybean cultivars

Fatty acid	Mean content (%)	Range of content (%)	Standard deviation	Coefficient variability (CV%)
Palmitic acid	11.00	8.2-13.5	1.06	9.65
Stearic acid	3.63	2.5-5.1	0.53	14.87
Oleic acid	21.47	16.9-27.3	2.53	11.73
Linoleic acid	53.99	46.9-58.3	2.19	4.11
Linolenic acid	9.93	6.6-13.2	1.53	15.43

Stearic acid is also a saturated fatty acid. The content ranged from 2.5 to 5.1%. The stearic acid content of 59 cultivars (84.2%) ranged from 3.0 to 4.5%. The coefficient variability was 14.87%

Oleic acid is an unsaturated fatty acid. It varied significantly among the different cultivars. The oleic acid content ranged from 16.9 to 27.3%. The oleic acid content of 47 cultivars (67.1%) ranged from 18.6 to 23.0%. The coefficient variability was 11.73%

Linoleic acid is an unsaturated fatty acid with two double bonds. Linoleic acid is an essential fatty acid for humans. Linoleic acid content indicates the nutrient value of soybean oil. Linoleic acid content varied from 46.9 to 58.3% for the 70 cultivars, and 59 of the cultivars (84.2%) had an acid content of 51.0 to 55.6%. The coefficient variability was 4.11%.

Linolenic acid is an unsaturated fatty acid with three double bonds. The three double bonds make the acid easily oxidized. Thus, the higher the linolenic acid content, the shorter the oil storage period. Therefore, the content of linlenic acid should be controlled to some lower level for a longer storage life. The linolenic acid content ranged from 6.6 to 13.2%. The content of linolenic acid of 57 cultivars (81.4%) ranged from 8.0 to 12.5%. Another 9% of the cultivars contained lower than 7.9% of the acid. The coefficient variability was 15.43%.

These results showed that there were significant differences in the contents of fatty acids among the cultivars. Among the 70 cultivars cultivated in Heilongjiang Provinces, there are 10 cultivars containing more than 51.0% linoleic acid and lower

than 8.0% linolenic acid. They were Nengfeng 10, Heinong 32, Muffing 5, Nengfeng 1, Nengfeng 9, Hongfeng 3, Hefeng 26, Hefeng 25, Hefeng 29, and Heinong 26.

References

Bao, Xu, et al. 1985. Jilin Agricultural Science 2:92.

Guoxun, Wang, et al. 1979. Chinese Oil Crop 1:46-49.

Mingxiang, Hu, et al. 1986. Jilin Agricultural Science 1:12-17.

Wilson, R.F., et al. 1981. Crop Science 21:788-791.

Burton, J.W., et al. 1983. Crop Science 23:744-747.

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Studies on chemicals controlling breeding in soybeans: Response of various genotypes on plant growth regulator

The response of seedling growth, agronomic traits and seed yield of various soybean genotypes to paclobutrozal, a plant growth regulator, were studied. The growth of root and stem of soybean seedling were inhibited by paclobutrozal. The inhibition rates of root growth of some genotypes were larger than those of other genotypes, but the inhibition rate of stem growth may be smaller than others. Paclobutrozal effects on root length, stem length and root and stem length were significant between genotypes. The influence of paclobutrozal on plant height and other agronomic traits of various genotypes were different and there was a significant correlation in seed yield between genotype and paclobutrozal treatment. The results indicated that the response of seedling growth, agronomic traits and seed yield of various soybean genotypes by plant growth regulator was very different. The new high yield cultivars which might depend on a plant growth regulator would be breeded with a chemically controlled breeds of soybeans.

Scientists have paid attention to research on the application of plant growth regulators (PGR's) in soybean production. Anderson et al., (1965) studied the response of soybeans to 2,3,5-triiodobenzonic acid (TIBA) under field conditions (Greer, 1965) and found that plant growth was inhibited, lodging decreased and seed yield increased when TIBA was sprayed at flowering. After this, many new PGR's, such as 2,4-D, GA, NAA, 7841, cycocel and BTS44-584 were used in soybean production, and some researchers found that the effects of PGR's on increasing soybean yield were unstable and varied in different cultivars (Anderson and Guanqin, 1983; Caldwell, 1973).

Paclobutrozal (PP333) is a new kind of PGR which retards growth, inhibits stem elongation and increases lodging resistance (Lianan and Qizhen, 1985). It's mechanism of activity might be caused by the accumulation of endogenous ABA and the inhibition of GA biosynthesis (Ling and Ruichi, 1988). This PGR was being used in soybean production (Yuhua, 1982). PGR's could directly or indirectly regulate the biosynthesis

and metabolism of endogenous hormones. The effects of PGR's may be affected by differences in the biosynthesis and metabolism of various genotypes. In contrast, the effect of PGR's may be stimulated by changing the level of the endogenous hormones with genetic improvement. The object of this thesis is to study the response of seedling growth and seed yield of various soybean genotypes to plant growth regulator. A new selective breeding method, Chemical Controlling Breeding in Soybeans, will be suggested.

Materials and Methods

I. Materials

Six summer soybean varieties in southern China, Zhongdou 24, Houzimao, You 88-25, You 91-1, You 91-3, and You 85-26, and seven same type varieties, Zhongdou 24, Houzimao, You 91-3, You 91-12, You 88-25, You 85-26 and You 86-305, were used in experiment I and II, respectively.

II. Methods

Experiment I. The effect of paclobutrozal on seedling elongation was tested by paper towel germination, in which seedlings were cultured in 0, 2, 5, 10, 20, 40, and 50 ppm paclobutrozal solution with two replications in 25 °C for seven days. Seedlings were exposed to light for 8 hours a day. The length of root and stem of 5 plants for every treatment of various genotypes in every replication were examined.

Experiment II. The response of seed yields and agronomic traits of various genotypes on paclobutrozal were tested in two treatments (0 and 150 ppm paclobutrozal) with two replications. After harvesting, the plant height, number of node, number of branch, number of pod, number of seeds per plant and 100-seed weight were examined.

III. Statistical treatment

The variance analyses for the length of stem and root in experiment I and seed yield in experiment II were made on a mixed model for testing the significance of genotype by paclobutrozal interaction (Yuhua, 1982). The inhibition rate of paclobutrozal on stem and root elongation was calculated.

Results

I. The response of seedling growth of various soybean genotypes treated with paclobutrozal

The seedling growth of six genotypes were all inhibited when they were cultured in paclobutrozal solution (Table 1). The higher the paclobutrozal concentration in solution, the higher the rate of inhibition. When paclobutrozal content in culture solution was lower than 10 ppm, inhibition rates increased greatly with increased paclobutrozal concentrations. In solutions with 2 ppm paclobutrozal, the mean inhibition rate of stem was 17.3%, but it was higher than 63% in 10 ppm paclobutrozal treatment. When the content was over 10 ppm, the inhibition rate increased at lower rates with the increase in paclobutrozal concentration. When paclobutrozal concentration was 50 ppm, the mean inhibition rate of stems of the six genotypes was 75.3%, 11.4% higher than that in 10 ppm. And it could be found that the inhibition rate of root was lower than that of stem.

Table 1. Inhibiting rate (%) of paclobutrozal to seedling growth of various genotypes in soybeans

Traits	Treatment (ppm)	2	5	10	20	30	40	50
root + stem	mean	13.4	30.7	42.2	44.4	50.1	51.4	57.4
	range	5.1-19.4	19.6-34.7	40.5-44.9	37.4-48.8	43.5-54.4	46.4-54.7	51.3-63.4
root	mean	8.0	11.6	15.6	15.9	24.5	26.5	35.6
	range	0-13.8	4.1-19.0	13.9-17.7	8.8-25.3	21.2-26.6	21.8-36.4	27.0-46.9
stem	mean	17.3	46.5	63.9	68.1	70.7	71.8	75.3
	range	0.9-30.7	22.0-55.6	59.1-67.1	56.0-75.2	61.9-73.4	69.7-72.0	66.6-79.7

In addition, the responses of various soybean genotypes to paclobutrozal were significantly different. The inhibited rate of seedling growth for various genotypes were different at various concentrations as seen where the range of inhibition in stem at 2 ppm paclobutrozal treatment was 0.9 to 30.7% (Table 1). The paclobutrozal treatment by genotype interaction varied not only in stem length but also in roots were significant (Table 2).

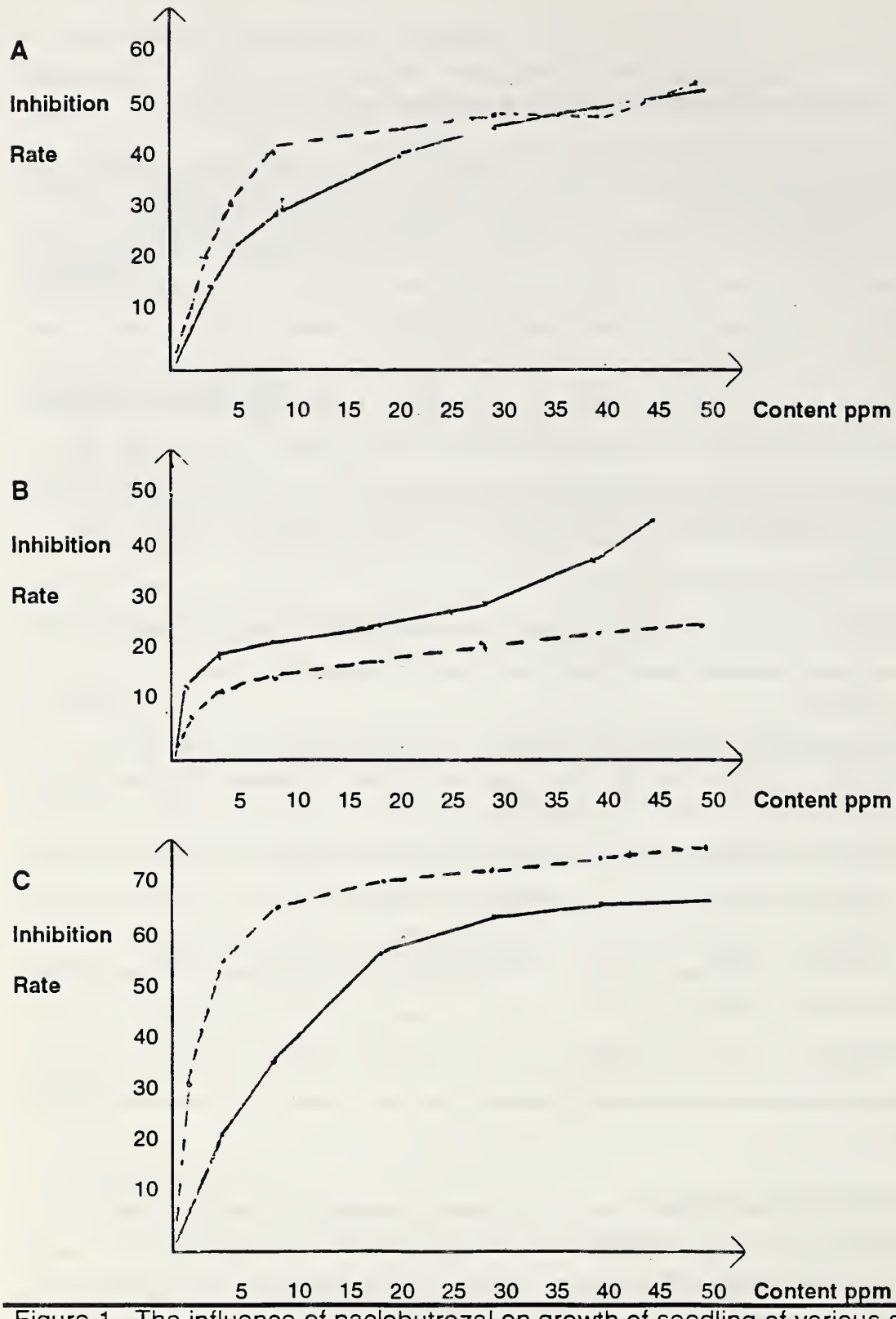

Figure 1. The influence of paclobutrozal on growth of seedling of various soybean genotyypes

A. stem + root B. root C. stem

You 88-25 ———— Zhongdou 24 – – – – – –

The inhibition rates of various paclobutrozal concentrations on two genotypes are indicated in Figure 1. In addition, it was found that the inhibition rate of stem of Zhongdou 24 was higher than that of You 88-25, but the inhibition rate of root of Zhongdou 24 was lower than that of You 88-25. This character could be used in soybean improvement by breeding a new variety in which root growth was inhibited at a lower degree but stem growth at a higher degree.

Table 2. Genotype by paclobutrozal interaction variance of seedling growth in soybeans

Source\traits	Length of root + stem		Length of root		Length of stem	
	MS	F	MS	F	MS	F
Genotype	13.05	14.09*	7.84	23.92*	10.81	19.77*
treatment	511.38	551.97*	32.59	99.41*	306.18	560.13*
treatment x genotype	2.89	3.12*	0.81	2.47*	1.98	3.63*
error	0.93		0.33		0.55	

* significant at 1% probability level

II. The response of seed yield of various soybean genotypes based on paclobutrozal concentration

When 150 ppm paclobutrozal were applied at flowering, soybean seed yield was obviously changed (Table 3 & 4). The mean yield of treatments was 21.95 (g), but the mean yield of control was 19.17 (g), the former was increased 14.5 % more than later. Table 3 and Table 4 show that the response of seed yield of various genotypes on paclobutrozal was very different, the range of yield of treatment against control was from -27.4% to +59.9%, and the treatment by genotype interaction was very significant. Because of this, on the one hand it was necessary to study the effect of paclobutrozal on released cultivar, on the other hand it would be possible to breed some new high yield cultivars which were dependent on some growth regulators.

III. The influence of paclobutrozal on agronomic traits of various soybean genotypes

Table 5 shows that there was little influence of paclobutrozal on the number of nodes per plant and the response of various genotype was similar, but the response of

other agronomic traits to paclobutrozal were different in genotypes. For example, the number seed per plant of You 91-12 was 94.74 in control plot, but was over 143.75 in treatment, later increased 49.01 more than the former. The 100-seed weight was 22.25 (g) in control plot and 20.24 (g) in treatment plot and the treatment decreased 2.01 (g) less than control. In contrast, the number seed per plant of You 91-3 was 190.94 in control plot, but was 129.04 in treatment, later decreased 61.90 less than the former. The 100-seed weight was 18.57 (g) in control plot and 19.26 (g) in treatment plot. The treatment increased 0.69 (g) more than the control. But the number seed of per plant and 100-seed weight of You 88-25 or Houzimao in treatment plot were all higher than that in the control plot.

Table 3. Genotype by paclobutrozal interaction variance of seed yield in soybeans

Source	df	SS	MS	F	F 0.05	F 0.01
genotype	6	273.54	45.60	39.23*	3.00	4.82
replication	2	10.58	5.29	4.55**	3.88	6.93
treatment	1	54.04	54.04	46.50*	4.75	9.33
genotype x treatment	6	155.70	25.95	22.33*	3.00	4.82
error	12	13.94	1.16			
total	27	507.80				

* significant 5% probability level
**significant 1% probability level

Discussion

To improve soybean yields, on one hand germplasm or new mutants could be used to expand the genetic basis of soybean breeding, on the other hand some new breeding methods (including selecting methods) could be created to increase the effect of resources used in soybean breeding. Soybean chemical breeding was a new selecting method which was based on the research results in modern plant physiology, especially in plant hormones and regulating of plant growth. In this selection method, artificial plant growth regulator (PGR) would be used in selecting generations in cross breeding. The genetic difference in the biosynthesis and metabolism of plant hormones

would be utilized and some new cultivars which were dependent on the PGR's would be breed. The object of this method was to expand the methods using soybean genetic resources, increase the effect of PGR's, and raise the soybean yield per unit area. The plant growth regulator was used as a selecting condition rather than a mutagen in this breeding method.

Table 4. The influence of paclobutrozal on seed yield (g) of various soybean genotypes

Genotype	Yield of treatment (1)	Yield of control (2)	(1-2)/2 x 100%
You 86-305	29.80	25.80	+15.50*
You 85-26	22.85	18.20	+25.55**
Houzimao	19.40	18.10	+7.18
You 88-25	25.00	17.00	+47.06**
You 91-12	22.70	14.20	+59.86**
You 91-3	15.10	20.80	-27.40**
Zhongdou 24	18.80	20.10	-6.47
Mean	21.95	19.17	+14.50**

* significant 5% probability level
**significant 1% probability level

Table 5. The influence of paclobutrozal on agronomic traits of various soybean genotypes

Genotype	Traits	Plant height	No. node	No. branch	No. pod	No. seed	100-seed weight (g)
You 86-305	control	90.5	18.3	4.4	73.0	154.03	23.25
	treatment	82.4	18.3	5.2	81.4	175.82	25.38
You 85-26	control	77.2	16.8	3.4	53.6	128.10	19.10
	treatment	72.6	16.6	4.0	76.2	185.17	19.98
Zhongdou 24	control	56.3	12.8	3.2	69.3	166.32	15.43
	treatment	54.4	12.7	2.5	75.3	184.49	15.43
Houzimao	control	61.6	15.3	3.2	60.9	127.28	17.49
	treatment	62.0	16.3	3.2	72.4	143.40	18.35

Table 5. continued

Genotype	Traits	Plant height	No. node	No. branch	No. pod	No. seed	100-seed weight (g)
You 88-25	control	79.3	15.5	2.6	49.1	97.71	23.76
	treatment	74.5	15.1	2.3	72.3	127.97	28.28
You 91-12	control	82.5	17.4	7.9	38.2	94.74	22.25
	treatment	85.1	17.4	8.5	57.5	143.75	20.24
You 91-3	control	84.9	17.9	6.0	88.4	190.94	18.53
	treatment	93.8	17.9	5.8	64.2	129.04	19.26

In this thesis, the concept of soybean chemical controlled breeding was suggested and the response of various genotypes to a plant growth regulator were studied. For soybean chemical controlled breeding, it would be necessary to study the genetics of susceptibility of soybeans to plant growth regulators, selecting method and breeding effect.

References

Anderson, I.C. and He Quanqin. 1983. The use of growth regulators on soybean. Chinese Agricultural Science. 3:27-31.

Caldwell, B.B. 1973. Soybeans: Improvement, Production, and Uses. American Society of Agronomy, Inc. pp. 239-240.

Greer, H.A.L. and I.C. Anderson. 1965. Response of soybean to triiodobenzoic acid under field conditions. Crop Sci. 5:229-332.

Liao Lianan and Guo Qizhen. 1985. A new plant growth regulator and germicide-paclobutrozal. Plant physiology communications. 6:56-58.

Li Ling and Pan Ruichi. 1988. Effect of PP333 on endogenous ABA and GA level in the leaves of Arachis hypogaea L. seedlings. Oil Crops of China. 2:36-39.

Ma Yuhua. 1982. Experiment Statistics. Agriculture Publishing House.

Yuan Xinfa et al. 1990. Effects of paclobutrazol on yield of spring soybean interplanted in wheat. Oil Crops of China. 3:86-88.

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New soybean cultivars selected in mendeleum for the region of southern Moravia

The process of selection of new cultivars was started in 1975 by formatting a collection of genotypes from Europe and overseas. This process still continues. First collections have been undergoing an intensive and complex evaluation from the point of view utility features, health conditions, reproductive ability in different regions etc. In 1980-1990 onto the best of them a radioselection had been applied (Gammacell 220, X-rays up to 40 kR, radiomimetics EMS up to 0.25% and MNU up to 1.5 mM with expositions 6, 12, and 24 hours). At the same time, in 1980, a crossing of distinct genotypes was started. According to the increase of efficiency of hybridization the volume of radioselection has been decreasing.

In 1990 field trials with new cultivars started to submit the best of them, 4517/92 and 4518/92 to preliminary state trials in 1993. It was followed by submitting new cultivars to preliminary state trials in 1994 - four cultivars in the Czech Republic (1395/93, 1396/93, 1387/93, 1389/93) and six in the Slovak Republic (as above plus 4517/92, 4518/92).

Table 1 shows the actual collection of the best genotypes in Mendeleum (as a leading institution for soybean breeding in the Czech Republic) together with information of their formatting. Genetic (morphologic) markers used in successful identification in F_1 population after hybridization are also described (some of them are used in biotechnology programs). Particulars are discussed in Kadlec & Wolf (1984), Kadlec & Vozda (1985), Kadlec et al., (1990, 1994, 1995), Vachunova & Wolf (1985, 1990).

Results of state preliminary trials in 1994 are shown in Table 2 and Table 3.The trials were performed at three breeding stations in the Czech Republic (A1, A2, A3) with control varieties Rita and Polanka and at two stations in the Slovak Republic (B1, B2) with Polanka as a control variety. The results are being described according to traits and localities.

Table 1. Actual list of genetic resources for the selection of new cultivars and new cultivars in state preliminary trials in the Czech and Slovak Republics (••)

1	2	3	4	5	6	7
1	1	Wilkin's	SE	w1w1tt	Pk; Miv	
2	2	Dunajka	ICHM	w1w1tt	Miv	
3	3	MERIT	ICHM	W1W1tt		
4	4	Isz-13	IFM	w1w1TT	Miv	
5	5	Traverse	ICHM	w1w1tt	Pk; Miv	
6	7	Altona	SE	W1W1TT	Miv	
7	8	Schae 01	ICHM	W1W1TT		
8	12	Zora	ICHM	W1W1TT	Pk; Miv	
9	13	PKF-1	ICHM	W1W1TT		
10	14	M-1	ICHM	W1W1TT	Pk; Miv	
11	18	MIRA	IFM	W1W1tt	Miv	
12	20	Sluna	SE	w1w1TT	Pk; Miv	
13	21	Maple Arrow	ICHM	W1W1TT	Miv	
14	23	S-1346	ICHM	W1W1tt	Pk; Miv	
15	30	Ewans	ICHM	w1w1tt		
16	33	Polanka	SE	W1W1TT	Miv	
17	41	Semu 8107	ICHM	w1w1tt	Miv	
18	41	Semu 8107	IFM	w1w1TT	Miv	
19	43	Crusader	IFM	W1W1tt		
20	44	Maple Donovan	IFM	W1W1tt	Miv	
21	48	Dawson	ICHM	W1W1tt		
22	51	Chandor	ICHM	w1w1TT		
23	53••	(5 x 12) x (21 x 30)	HYB	w1w1TT	Pk; Miv	4517/92
24	54••	(23 x 30) x 1	HYB	w1w1tt	Pk; Miv	4518/92
25	155	GSU	SE	W1W1TT	Pk; Miv	
26	158••	44 x 8	HYB	W1W1TT	Pk; Miv	1387/93
27	159	1 x 18	HYB	w1w1tt	Pk; Miv	xx
28	181	48 x 43	HYB	W1W1TT	Pk; Miv	xx
29	196	Gadir	SE	W1W1tt	Miv	
30	197	Zefir	SE	W1W1tt		
31	219••	20 x 8	HYB	W1W1TT	Pk; Miv	1395/93
32	225	20 x 18	HYB	W1W1TT	Pk; Miv	xx

Table 1. continued

1	2	3	4	5	6	7
33	236**	20 x 8	HYB	W1W1TT	Pk; Miv	1396/93
34	242	23 x 21	HYB	w1w1tt	Pk; Miv	xx
35	243**	23 x 8	HYB	W1W1tt	Pk; Miv	1389/93

Key
1.List number
2-3.Initial number and title of genotype in Mendeleum's collection
4.Variety origin: SE - by selection; ICHM -by chemical mutagenesis; IFM - by physical mutagenesis; HYB - through crossing
5.Genetic markers: alleles TT and tt; W1W1 and w1w1
6.Application in in vitro cultures: Pk - anther cultures; Miv - micropropagation
7.Numerical description of new cultivars (descr. /year); xx- field trials

Table 2.Fundamental information of the pre-testing sites

I. Czech Republic - control varieties: Rita, Polanka

Site of pre-testing	A1-Nejdek	A2-U. Ostroh	A3-Lednice
Altitude	170 m	196 m	199 m
Rainfall I-XII/	540 mm	551 mm	525 mm
Rainfall I-X /	466	481 mm	455 mm
Rainfall I-X /94	354	368 mm	349 mm
Temperature	9.2	9.1 C	9.3 C
Soil unit	Luv. -hap. -bl.	Eut. camb.	Luv. -hap. -bl.
Previous crop	Winter wheat	Maize sil.	Winter wheat
Fertilizer	N 40 kg/ha	N 50 kg/ha	N 21 kg/ha
Sowing	2.V. 1994	3.V. 1994	30.IV. 1994
Spacing	37.5 cm	37.5 cm	37.5 cm
Harvest	gradually	gradually	gradually
Treatment	Maloran 3 kg/ha	Maloran 3 kg/ha	Afalon 2.5 kg/ha

II. Slovak Republic - control variety: Polanka

Site of pre-testing	B1-Topolníky	B2-Trnava
Altitude	112 m	175 m
Rainfall I-XII /	556 mm	562 mm
Rainfall I-X /	481 mm	495 mm
Rainfall I-XI /94	365	362 mm

II. Slovak Republic - control variety: Polanka		
Site of pre-testing	B1-Topolníky	B2-Trnava
Temperature	9.3 C	9.4 C
Soil unit	Eut. camb	Luv. -hap. -bl.
Previous crop	Maize	Maize
Fertilizer	N 40 kg/ha	N 40 kg/ha
Sowing	30.IV. 1994	2.V. 1994
Spacing	37.5 cm	37.5 cm
Harvest	gradually	gradually
Treatment	Afalon 3kg/ha	Afalon 3kg/ha

Table 3 Pre-testing report of soybean cultivars in 1994, evaluated traits

	Traits										
Variety	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
					Site A1						
'1396/93'	2.79	109	156.6	8.0	8.8	7.0	95	14	71	80	137
RITA	2.56	100	179.6	8.0	8.8	5.0	78	12	69	79	128
'1389/93'	2.48	97	141.6	7.0	7.5	7.0	84	16	71	80	137
'1395/93'	2.33	91	146.1	8.0	8.3	7.0	111	15	71	86	136
'1387/93'	2.06	81	155.8	8.0	9.0	5.0	61	10	69	79	125
					Site A2						
'1387/93'	2.11	130	234.3	9.0	4.5	9.0	77	6	73	104	153
'1395/93'	1.87	115	191.0	9	4.0	9.0	73	8	66	102	151
RITA	1.62	100	262.1	9	8.0	9.0	75	6	62	99	151
'1389/93'	1.42	88	237.8	9.0	8.8	9.0	59	5	68	99	135
'1396/93'	1.18	73	218.0	9.0	5.3	9.0	68	6	68	104	150
					Site A3						
'4517/92'	3.35	152	154.8	9.0	8.1	9.0	82	13	72	88	139
'4518/92'	3.03	137	135.1	9.0	9.0	9.0	81	14	73	89	141
'1396/93'	2.98	135	153.1	8.0	8.7	7.5	95	13	70	82	140
'1389/93'	2.96	134	140.2	8.0	8.0	8.0	83	16	70	81	139
'1395/93'	2.73	124	122.8	8.0	8.5	7.5	115	16	72	88	138
'1387/93	2.54	115	154.2	8.0	9.0	6.0	63	11	70	80	126
POLANKA	2.21	100	134.2	8.0	9.0	6.0	80	12	67	79	129

Table 3.continued

					Site B1						
'4517/92'	3.01	174	156.9	9.0	8.5	9.0	85	14	73	87	145
'1396/93'	2.96	171	156.3	9.0	8.8	8.0	99	15	75	88	144
'1389/93'	2.88	166	142.1	8.1	8.2	8.0	84	15	72	83	142
'4518/92'	2.73	158	138.8	9.0	9.0	9.0	85	14	76	90	147
'1387/93'	2.47	143	157.1	8.0	9.0	7.0	66	12	72	82	131
'1395/93'	2.34	135	128.8	8.5	8.7	8.0	119	16	77	90	142
POLANKA	1.73	100	136.2	8.5	8.0	7.0	83	13	69	80	131
					Site B2						
'4517/92'	1.62	103	153.6	9.0	9.0	9.0	83	13	74	85	143
'1396/93'	1.61	102	155.5	9.0	8.9	8.2	97	13	73	87	142
'1389/93'	1.75	111	141.9	8.0	8.0	8.0	88	15	73	81	140
'4518/92'	1.95	123	137.1	9.0	9.0	9.0	83	14	75	88	149
'1387/93'	2.18	138	155.2	8.0	8.8	7.5	68	13	71	80	130
'1395/93'	1.81	115	130.1	8.0	8.0	8.0	113	17	76	88	140
POLANKA	1.58	100	137.2	8.0	7.8	7.0	79	12	70	77	129

Evaluated traits
1 -Yield of grain [t/h]
2 -Yield of seeds [%]
3 -Weight of one thousand seeds [g]
4 -Pseudomonas [scale 9-1]
5 -Standing ability [scale 9-1]
6 -Resistance to pod opening [scale 9-1]
7 -Height of plant [cm]
8 -1st pod position height [cm]
9 -Days from sowing to full flowering [days]
10 -Days from sowing to full setting pod [days]
11 -Days from sowing to full maturity [days]

Conclusion

The aim of the short report submitted was to describe highlights in the process of selection new soybean cultivars in Mendeleum as a leading institution for soybean breeding in the Czech Republic. The presented results seem to be very optimistic for future.

References

Kadlec, M. and J. Wolf. 1984. Influence of forced mutation and selection in mutual associations between quantitative traits in filial generations of soybean (IV); Acta Univ. Agr. Fac. Agr. 32(4):323-329.

Kadlec, M. and J. Vozda (1985. Genetic analysis of soybean populations. Research report; Univ. Agric. Brno, Mendeleum Lednice na Morave.

Kadlec, M. et al. 1990. Formation of soybean ideotype. Research report; Univ. agric. Brno, Mendeleum Lednice na Morave.

Kadlec, M. et al. 1994. Genetic markers and their association to quantitative traits. Research report funding by Czech University Fund, Prague, Mendeleum Lednice na Morave.

Kadlec, M. et al. 1995. Formation of genetic resources of soybean; manipulations in vitro. Final research report, Univ. Agric. Brno, Mendeleum Lednice na Morave.

Vachunova. A. and J. Wolf. 1985. Genetic analysis of soybean population structure according to quantitative traits and theoretical bases of selection. Final research report, Univ. Agric. Brno, Mendeleum Lednice na Morave.

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Associations between morphological characteristics in F_2 populations of soybean

In the case of nominal data (senzoric evaluation, morphological or biochemical markers, qualitative characteristics like degree of earliness or lodging, affection by deseases, etc.) it is often desirable to know whether the knowledge of one nominal variable gives some advantage in the process of breeding.

As an initial material was F_2 generation after crossing of genotypes Glycine max (L.) Merril x Glycine soja ssp. ussuriensis, from the collection in Mendeleum. Cultural species Glycine max (L.) Merril, is morphologically very diversible with height within a scale 0.3-2 m. On the other hand Glycine soja ssp. ussuriensis, is creeping with narrow leaves and fine-grained. For each F_2 plant the following characters were measured: T-type of trychom's tips, DS -density of trychoms on stems and branches, degree of earliness and lodging. T and DS characters have served as morphological markers.

To measure the association between markers T, DS and nominal variables lodging, earliness two approaches have been applied:

1) Contingency tables for testing the significance of association between nominal variables. They are based on χ^2 statistics. The results are shown in Tables 1 - 3. Cramer's V ($0 \leq V \leq 1$) and C contingency coefficient describe the level of association and they are defined as:

$$V = \sqrt{\frac{\chi^2}{N * \min(r-1, s-1)}}, \quad C = \sqrt{\frac{\chi^2}{\chi^2 + N}}$$

where $r \cdot s = N$ is a dimension of table (row columns). Unfortunately, two different tables can be compared only in the case of the same dimension. To break this obstacle we used a second approach:

2) The notion of entropy can be used as a measure of association two variables A and B. This measure is independent on table's dimension. The entropy U ($0 \leq U \leq 1$) is defined as:

$$U = 2\left(\frac{H(A) + H(B) - H(A,B)}{H(A)+H(B)}\right)$$

where

$$H(A) = -\sum_i p_i^A \ln p_i^A ; H(B) = ; -\sum_i p_i^B \ln p_i^B ,$$

$$H(A, B) = -\sum_i \sum_i p_{ij} \ln p_{ij}$$

and

$$p_i^A = \frac{r_i}{N}, \; p_j^B = \frac{s_j}{N}, \; p_{ij} = \frac{n_{ij}}{N}$$

(r_i, s_j, n_{ij} are row, column and cell frequencies in contingency table).

On the other hand the last approach doesn't serve as a measure of significance - it is desirable to use both of them (χ^2 statistics for testing significance of association and entropy U for comparisons different tables).

Figure 1 shows the entropy U for all characters measured in F_2 population and a graphical scheme compared real and theoretical frequencies in the case of couple DS x Earliness. Statistical significance was proved only for two couples DS x Earliness and DS x Lodging (see Tables 2-3). It follows that density of trychoms could play a role of a marker in selection strategies. In fact, because χ^2 statistics used in tests doesn't estimate a recombinant coefficient in crossing-over events, the level of association is underestimated. Thus, type II error could be higher. Nevertheless, the methods presented above are applicable especially in analyses of categorial data.

Combination	Entropy U
T x Earliness	0,03
DS x Lodging	0,08
DS x Appearance	0,06
DS x Earliness	0,09
W x T	0,01
Lodging x Appearance	0,11
Lodgng x Earliness	0,06
Appearance x Earliness	0,07

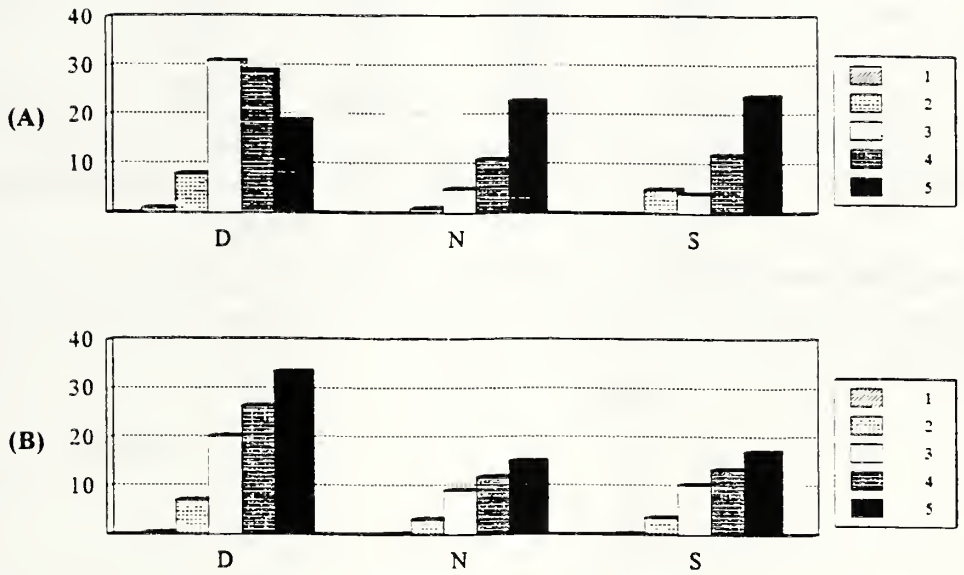

Figure 1: Values of entropy U for pairs of morphological markers DS (density of trychoms: D-dense, N-normal, S-sparse), T (type of trychoms), W/w (flower colors: W-pink, w-white) with qualitative characteristics like degree of earliness (1- very late, 5 - very early), lodging and a whole appearance. For a couple DS x Earliness there is shown a graphical comparison of real (A) and theoretical (B) frequences.

Combination	Entropy U
T x Earliness	0,03
DS x Lodging	0,08
DS x Appearance	0,06
DS x Earliness	0,09
W x T	0,01
Lodging x Appearance	0,11
Lodging x Earliness	0,06
Appearance x Earliness	0,07

Table 1: Contingency table and entropy U for characters T x Earliness (T = type of trychoms: R - round, S -sharp tips)

Frequency/	Earliness					
Theor.value/	very late <--> very early					
Percentage	**1**	**2**	**3**	**4**	**5**	Total
R	2	36	64	16	11	129
	4.5	38.8	58.2	17.2	10.4	
	1.16	20.81	36.99	9.25	6.36	74.57
S	4	16	14	7	3	44
	1.5	13.2	19.8	5.8	3.6	
	2.31	9.25	8.09	4.05	1.73	25.43
Total	6	52	78	23	14	173
	3.47	30.06	45.09	13.29	8.09	100.00

Statistics	**d. f.**	**Value**	**Significancy level** α
χ^2	4	8,89	0,06
Contingency coefficient		0,22	
Cramer's V		0,23	
Entropy U		0,03	

Table 2: Contingency table and entropy U for characters DS x Earliness (DS = density of trychoms: D -dense, N - normal, S - sparse)

Frequency/	Earliness					
Theor.value/	very late <--> very early					
Percentage	**1**	**2**	**3**	**4**	**5**	Total
D	5	34	42	7	0	88
	3.1	26.5	39.7	11.7	7.1	
	2.89	19.65	24.28	4.05	0	50.87
N	1	9	20	5	5	40
	1.4	12	18	5.3	3.2	
	0.58	5.2	11.56	2.89	2.89	23.12
S	0	9	16	11	9	45
	1.6	13.5	20.3	6	3.6	
	0	5.2	9.25	6.36	5.2	26.01
Total	6	52	78	23	14	173
	3.47	30.6	45.09	13.29	8.09	100.00

Statistics	**d. f.**	**Value**	**Significancy level** α
χ^2	8	30,68	0
Contingency coefficient		0,39	
Cramer's V		0,3	
Entropy U		0,09	

Table 3: Contingency table and entropy U for characters DS x Lodging (DS = density of trychoms: D - dense, N -normal, S - sparse)

Frequency/	Lodging					
Theor.value/	erect form <--> twine form					
Percentage	**1**	**2**	**3**	**4**	**5**	Total
D	1	8	31	29	19	88
	0.5	7.1	20.3	26.5	33.6	
	0.58	4.62	17.92	16.76	10.98	50.87
N	0	1	5	11	23	40
	0.2	3.2	9.2	12	15.3	
	0	0.58	2.89	6.36	13.29	23.12
S	0	5	4	12	24	45
	0.3	3.6	10.4	13.5	17.2	
	0	2.89	2.31	6.94	13.87	26.01
Total	1	14	40	52	66	173
	0.58	8.09	23.12	30.6	38.15	100.00

Statistics	**d. f.**	**Value**	**Significancy level** α
χ^2	8	28,07	0
Contingency coefficient		0,37	
Cramer's V		0,29	
Entropy U		0,08	

Reference

Kadlec M., J. Létal, and B. Janousek. 1994. Screening of morfological and biochemical markers - Glycine sp.; Zahradnictví 21(3):201-211.

Létal J. 1995. Methods Evaluating the Association between Marker Loci and Quantitative Traits. J. Scripta, Masaryk University Press, Brno (in press).

Press W.H., B.P. Flannery, S.A. Teukolsky, and W.T. Vetterling. 1990. Numerical Recipes in Pascal - The Art of Numerical Computing; Cambridge University Press.

SAS/STAT: Guide for personal computers. 1987). Version 6, SAS, Cary, NC.

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Lodging habit in commercial varieties of soybean released in India

Adequate lodging resistance is an important, desirable character for the development of improved varieties of soybean. Severe lodging causes significant yield loss (Leffel, 1961; Cooper, 1971a, 1971b). The present study reports the lodging habit of 46 prominent soybean varieties releabed for commercial cultivation in India.

46 soybean varieties were grown during rainy seasons of 1991 and 1993 at the research farm of National Research Centre for Soybean, Indore (22° 41' N latitude, 75° 52' E longitude and 540 meters altitude). Observations were recorded on mature plant height, lodging habit and growth habit. The lodging habit of the Soybean plants was rated on a scale from 1 to 5, with 1 for erect plants, 3 for plants that are leaning at a 45° angle and 5 for prostrate plants .

Mean data on plant height , lodging score and growth of the varieties were presented in Table 1. Varieties were arranged in the increasing order of their plant height. Lodging score and plant height showed a strong positive correlation (r = 0.60; significant at 0.01 P). Tall varieties and especially those with indeterminate or semi-indeterminate growth habit were more prone to lodging. Determinate type varieties recorded lower lodging score. The present study indicates that tall varieties with semi-erect plant types viz. Pusa 40, Pusa 16, Pusa 22, Hardee, Ankur, Pusa 20, Pusa 24, PK 416 and PK 564 may also be suitable for mechanical harvesting. Varieties Pusa 40, PK 416, Pusa 16, PK 564 and Pusa 24 possessed the additional advantage of sufficient gap between the ground level and the point of insertion of the lowest pod for efficient mechanical harvesting (Karmakar et al., 1994).

Table 1. Lodging score, growth habit and plant height in Indian soybean varieties

Variety	Growth habit	Plant height (cm)	Lodging score
JS 71-05	Determinate	45.6	1.0
JS 2	"	53.3	1.0

Table 1. continued

Variety	Growth habit	Plant height (cm)	Lodging score
Shilajeet	Determinate	53.4	1.0
VLS 2	"	57.3	2.0
VLS 1	"	59.7	1.0
JS 335	Semiindeterminate	60.0	5.0
PK 472	Determinate	62.1	1.0
Shivalik	"	63.3	1.4
Birsa soy I	"	63.9	4.0
NRC 2	"	66.3	1.0
PK 327	"	67.3	1.4
Alankar	"	69.2	2.7
Monetta	"	70.3	1.7
PK 471	"	71.0	1.0
PK 262	"	71.2	1.0
PK 308	"	72.0	1.0
Bragg	"	72.5	1.0
PK 564	"	72.6	1.0
MACS 13	Semiindeterminate	75.3	4.0
PuBa 37	Determinate	76.0	5.0
JS 76-205	Semiindeterminate	77.3	4.0
PK 416	Determinate	77.6	1.4
Pusa 24	"	79.1	2.1
Pusa 20	"	79.4	2.0
Pusa 16	Semiindeterminate	80.6	2.0
Durga	"	82.4	3.7
MACS 57	"	82.6	5.0
Gaurav	Determinate	84.4	4.0
Ankur	"	84.6	2.0
Punjab I	Semiindeterminate	85.6	5.0
KHSb 2	"	88.1	5.0
JS 80-21	Determinate	89.3	3.0
MACS 124	Semiindeterminate	93.0	5.0
Improved Pelican	Indeterminate	95.8	4.0

Table 1. continued

Variety	Growth habit	Plant height (cm)	Lodging score
Gujarat soy 1	Indeterminate	95.7	5.0
Hardee	Determinate	98.6	2.4
T 49	Indeterminate	100.1	5.0
MACS 58	Semiindeterminate	100.2	4.0
Gujarat soy 2	Indeterminate	100.7	4.0
Kalitur selection	Determinate	101.6	3.4
Pusa 22	"	102.9	2.7
JS 75-46	Semiindeterminate	103.6	4.4
Lee	"	104.6	4.0
Co 1	Indeterminate	106.5	5.0
Kalitur	"	107.1	5.0
PuBa 40	Determinate	111.3	3.0

References

Cooper, R.L. 1971a. Influence of early lodging on yield of soybean [Glycine max (L.) Merr.]. Agron. J. 63:449-450.

Cooper, R.L. 1971b. Influence of soybean production practices on lodging and seed yield in highly productive environments. Agron. J. 63:490-493.

Leffel, R.C. 1961. Plant height as a selection criterion in soybean breeding. Crop Sci. 1: 346-349.

Karmakar, P.G., S.P. Tiwari and V. Kumar. 1994. Seed filling duration and height of insertion of the lowest pod in Indian soybean varieties. Soybean Genet. Newsl. 21:104-107.

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Variability, heritability and character association in M_3 families of gamma irradiated soybean

Recent spurt in soybean popularity in India is due to the commercial value of its various by-products as well as number of advantages associated with soybean oil e.g. reducing heart diseases, gout and rheumatism. Soybean research and development in the recent past resulted in alarming growth of soybean cultivation. Further major steps in soybean improvement bedsides striving for better seed germination and minimizing pod shattering was concentrated for development of varieties relatively free from linolenic acid, lipoxygenase enzyme and trypsin inhibitors (Prasad, 1994).

Defect rectification in soybean varieties for their improvement has been possible through mutagenesis (Bhatnagar and Tiwari, 1991). Since a negative association between oil and protein is well established, we attempted mutation breeding program in soybean with a view to combine high oil and protein existing well adopted genotypes.

Material and methods

Dry seeds of the varieties were treated with gamma ray doses (10, 20, 30, 40, and 50 kR). In M_2 and M_3 generations both micro and macro mutations were obtained. Single plant from M_2 generations were selected discarding agronomically undesirable plants and their progenies were evaluated in M_3 generation. Differences for oil content in the mutant families and parental genotypes was determined by nuclear magnetic resonance (NMR) analysis. Ten uniform plants were selected from the central rows for recording observations. Analysis of variance and different genetic parameters (Table 1, a) were calculated as per Burton (1952). Singh and Chaudhari (1977) and Allard (1960). Genotypic coefficients and path coefficients were estimated as per Craxton and Cowden (1964) and Dewey and Lu (1959) respectively.

Results and Discussion

Analysis of variance showed significant differences among genotypes for all characters (Table 1). The coefficient of variation indicated only extent of variability present in different characters and did not indicate its heritable portion, which was further ascertained from the heritability estimates in broad sense which included both additive and non-additive gene effects (Hanson et al., 1956). Pods/plant had maximum GCV (600.025) followed by plant height (166.218).

Estimates of variability ranged from 26.20 (oil %) to 90.60% (days to 50% flowering) even though the characters days to 50% flowering and 100 grain weight had high heritability (90.60 and 77>30 respectively), it was accompanied by low CV values (8.349 and 4.257 respectively)> This might be due to the variation in environmental component involved in these traits.

Expected genetic advance (GA) ranged from 0.858 (oil %) to 22.505 (pods/plant). Pods/plant, plant height, yield/plant and days to maturity showed high GA with high GCV and should be considered for obtaining high genetic gain.

The results are in confirmation with Katariya and Sengupta (1972) Amarnatha et al., (1991) and Mahajan et al., (1994). Highly significant genotypic and positive correlations recorded between yield/plant and pods/plant (0.318): 100 grain weight (0.669) and branches/plant (.638). However, highly significant negative correlation between yield and days to 50% flowering (-0.365) and maturity (-0.607); plant height (-0.389) and oil content (-0.288) was noticed.

Further oil content was found to be highly significant and positively correlated with days to maturity (0.56) and branches/plant (0.336) while it was negatively correlated with plant height (-0.382).

Branches/plant were highly significant negatively correlated with days to flowering (-0.270); days to maturity (-0.407); plant height (-0.654) while positively and highly significant with 100 grain wt. (0.566) and pods/plant (0.815).

Highly significant negative correlations were also observed between pods/plant with days to 50% flowering (-0.518): days to maturity (-0.419) and plant height (-0.252) but positively correlated with 100 grain weight (0.511).

Further 100 grain weight was found to be highly negative and significantly correlated with days to 50% flowering (-0.573) and maturity (-0.758) and plant height (-0.519). While days to 50% flowering and maturity was found to be highly significant and positively correlated with days to maturity (-0.592).

Table 1. Analysis of Variance, genetic parameters of variation, correlation coefficients and path analysis in M_3 families of gamma irradiated soybean

Parameters	Days required for 50% flowering	Maturity	Plant height (cm)	100 grain height (gm)	Pods /plant	Branches /plant	Oil %	Yield /Plant
a) Analysis ofVariance: Source of M.S.S.								
1) Replication - 1	81.96	416.25	2127.5	29.629	8622.5	1.042	13.821	86.03
2) Genotypes - 74	159.10**	588.75*	2356.6*	75.491**	8678.8*	15.542*	31.935*	779.178**
3) Error - 74	7.88	106.74	968.7	9.656	3321.7	5.995	18.67	89.517
b) Genetic parameters								
1) Mean	53.75	108.93	62.90	11.957	100.82	4.542	23.08	11.301
2) C.V. %	5.22	9.48	20.64	25.98	67.16	53.90	18.72	83.72
3) Coefficient of variability (P)	7.561	22.601	69.346	3.292	267.855	0.477	0.663	34.483
4). Coefficient of variability (G)	8.349	33.27	166.218	4.257	600.025	1.077	2.530	43.435
5) Heritability (h^2) %	90.60	67.90	41.70	77.30	44.60	44.30	26.20	79.40
6) Genetic advance (G.A.)	5.393	8.068	11.075	3.285	22.505	0.947	0.858	10.779
7) Expected G.A. (% mean)	10.033	7.407	17.606	27.477	22.323	20.851	3.72	95.387

Table 1. continued

Parameters		Days required for 50% flowering	Maturity	Plant height (cm)	100 grain height (gm)	Pods /plant	Branches /plant	Oil %	Yield /Plant
c) Coefficience of correlationand path analysis[@]									
1) Days required for 50% flowering	rg[b]	1.000	0.592••	0.184	-0.573••	-0.518••	-0.270••	0.132	-0.365••
	p[c]	1.208	-1.381	-0.230	0.279	-1.123	0.726	0.157	-
2) Days required for maturity	rg[b]	-	1.000	0.170	-0.758••	-0.419••	-0.407••	0.560••	-0.607••
	p[c]	-2.232	0.715	-0.212	0.360	-0.909	1.095	0.667	
3) Plant height (cm)	rg[b]	-	-	1.000	-0.519••	-0.242•	-0.654••	-0.382••	-0.389••
	p[c]	-1.247	0.223	-0.397	0.252	-0.524	1.760	-0.455	
4) 100 grain weight(gm)	rg[b]	-	-	-	1.000	0.511••	0.566••	-0.127	0.669••
	p[c]	-0.487	-0.692	1.767	0.647	1.108	-1.524	-0.151	-
5) Pods/plant	rg[b]					1.000	0.815••	0.016	0.398••
	p[c]	2.167	-0.626	0.978	0.302	-0.249	-2.193	0.019	
6) Branches/plant	rg[b]						1.000	0.336••	0.638••
	p[c]	-2.692	-0.326	0.949	0.816	-0.276	1.766	0.400	
7) Oil %	rg[b]							1.000	-0.288•
	p[c]	1.191	0.160	-1.307	0.476	0.062	0.035	-0.904	
8) Yield/plant	rg[b]								1.000
	p[c]								

[@] Residual effect (1.062) = underlined figures denote direct effects, and • and •• = significant at 5 and 1 % level respectively.
M.S.S. = Mean sum of square
G = genotypic
P = phenotypic
rg[b] = genotypic correlation coefficient
p[c] = path coefficient

Path coefficient analysis revealed that, branches/plant (1.766) had largest positive direct influence on grain yield followed by days to 50% flowering (1.208), days to maturity (0.715) and 100 grain weight (0.647). The direct effect of pods/plant was negative but had positive significant correlation of this trait with grain yield could be due to high positive indirect effects through days to 50% flowering, plant height and 100-grain weight. Therefore characters viz. branches/plant 100-grain weight and pod/plant should be given due importance in selection programs for improving grain yield of soybean.

Thus, from the results it was indicated that characters viz. 100-grain weight, branches/plant and pods/plant were the important characters since they were positively and significantly associated with grain yield/plant, and hence they were reliable selection indices for M4 generation. Path analysis revealed highest positive direct effect of pods/plant on grain yield/plant as reported earlier by Rajput et al., (1986), Bargale et al., (1988) and Mehetre et al., (1994). Further 100-grain weight and branches/plant exerted x highest indirect effect through days to 50% flowering. On the basis of correlation and path analysis, it was revealed that the characters pods/plant, 100-grain weight and branches plant were the most important yield contribution characters hence should be given more emphasis while making selections from M_3 generation of irradiated material.

References

Allard, R.W. 1968. Principles of plant breeding. John Wiley and sons, New York, London, p. 89-98.

Amarnath, K.C.N., S.R. Vishwanatha and B.C. Chennakeshava. 1991. Phenotypic and genotypic correlation coefficients of some quantitative characters in soybean [Glycine max (L.) Merrill]. Mysore J. Agric. Sci. 24:445-449.

Bargale, M., S.P. Singh and M.C. Barsale. 1988. Path coefficient analysis for yield and its components under different sowing dates in soybean [Glycine max (L.) Merrill]. PKU Res. J. 12:61-63.

Bhatnagar, P.S. and S.P. Tiwari. 1991. Soybean improvement through mutation breeding in India. In. Plant mutation breeding for crop improvement. Vol. I. IAEA, Vienna, p. 381-391.

Bhatnagar, P.S., S.P. Tiwari and C. Singh. 1992. Disrupting the negative association between oil and protein content in soybean seeds through mutagenesis. Mut.

Breed. New. Letter. 37:7.

Hanson, G.H., Robinson, and R.E. Comstock. 1956. Biometrical studies of yield in segregating populations of Korean Lespedeae. Agron. J. 48:267-282.

Conger, B.V., L.W. Skinner and L.N. Skold. 1976. Variability for components of yield induced in soybeans by seed treatment with gamma radiation, fission neutrons, and ethylmethyl-sulphonate. Crop Sci. 16:233-236.

Dewey, D.R. and K.H. Lu. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. Agron. J. 51:515-518.

Kataria, A.S. and K. Sengupta. 1972. A study on the variability in soybean [Glycine max (L.) Merrill]. Indian Agri. 16(2):155-159.

Mahajan, C.R., P.A. Patil, S.S. Mehetre, and R.D. Ghatge. 1994. Genotypic and phenotypic variability and heritability of some quantitative characters in soybean [Glycine max (L.) Merrill]. Ann. Agric. Res., 15(1):41-44.

Mehetre, S.S., C.R. Mahajan, R.D. Ghatge and P.M Dhumal. 1994. Induced genetic variability and character association in soybean. Crop Res., 8(23):348-353.

Reasad, M.V.R. 1994. Oil seeds, growth should accelerate. The Hindu - In survey of Indian agriculture. p. 50-53.

Rajput, M.A., G. Sanwar and K.H. Tahir. 1986. Path coefficient analysis of development and yield components in soybean. Soybean Genetic Newsletter 13:87-91.

Singh, R.K. and B.D. Chaudhary. 1977. Variance and co-variance analysis. Biometrical methods in quantitative genetics analysis. Kalyani Publ. New Delhi. p. 38-69.

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Effect of foliar application of growth substances on growth and yield

Kolhapur district of Maharashtra state of India is well known for highest yield potential pocket of soybean cultivation.

In this area, soybean is grown mainly during rainy season. High humidity favors luxuriant growth of soybean varieties which creates difficulty in easy and uniform application of plant protection measures and penitrance of sunlight through plant canopy. Heavy lodging of plants occurs due to heavy rains accompanied by high wind velocity resulting in heavy economic losses. Since an alternative of foliar application of growth substances is suggested, we studied the effect of a few growth substances on the growth and yield of soybean under Kolhapur conditions.

Material and Methods

Four growth substances in three concentrations were applied in the form of foliar spray to a tall growing soybean variety. Two sprays were applied; one at floral initiation and second, seven days thereafter. Observations were recorded on various yield contributing character and yield (Table 1).

Results and Conclusions

Yield and ancillary data presented in Table 1 revealed that grain yield per net plot, per plant grain yield and 100 grain weight differences due to various treatments are statistically significant.

The foliar application of 100 ppm at floral initiation gave highest grain yield (3194 kg/ha) and was significantly superior to absolute control (1667 kg/ha) and water spray (1736 kg/ha) and rest of the treatments. Highest grain yield produced by this

treatment is mainly because of its superiority in reduction of plant height, grain yield per plant and 100 grain weight.

Table 1. Effect of growth regulating substances on growth and yield of soybean variety MAC-124: Yield and ancillary data.

Sr. No.	Treatments: Foliar application of	Yield kg/net plot	Yield kg/ha	Plant height cm Days after sowing: 72	92	at harvest	No. of branches /plant	No. of pods /plants	Grain yield plant 'g'	100 grain wt. 'g'
1	25 ppm MH	0.950	2638••	43.0	43.8	42.4	7.4	37.7	7.97	13.4
2	50 ppm MH	0.850	2361	45.2	44.0	45.5	7.6	27.2	6.59	12.5
3	100 ppm MH	0.875	2430	43.0	42.5	42.2	5.2	29.2	5.96	12.6
4	25 ppm CCC	0.725	2013	41.8	40.5	41.7	7.2	28.3	5.19	12.9
5	50 ppm CCC	0.950	2638••	41.4	42.5	41.7	6.6	27.9	6.55	13.5
6	100 ppm CCC	1.150	3194••	49.5	50.1	49.5	7.6	27.4	6.70	13.0
7	25 ppm GA	1.050	2917••	52.0	50.1	52.0	5.2	16.2	6.19	14.0
8	50 ppm GA	0.375	2430	56.7	59.4	56.7	5.8	22.9	4.10	12.5
9	100 ppm GA	0.900	2500••	65.5	63.9	66.2	6.6	23.4	5.29	12.3
10	25 ppm AA	0.675	1875	64.6	65.1	64.6	5.6	18.7	3.36	12.9
11	50 ppm AA	0.750	2083	43.4	56.0	58.0	6.6	26.4	4.56	13.1
12	100 ppm AA	0.725	2014	45.9	48.0	45.9	6.8	18.5	4.35	12.7
13	Water spray	0.625	1736	58.5	61.2	70.5	6.7	32.5	4.07	11.7
14	Absolute control	0.600	1667	56.5	62.6	73.0	6.8	27.2	4.01	12.0
	SE ±	0.063	175.0			4.827			0.707	0.165
	CD at 1%	0.268	774.4••			20.558••			2.159•	0.702••
	CV %		10.71			12.73			18.69	1.83

Foliar application of 25 ppm as well as 50 ppm CCC reduced the plant height significantly (41.7 cm) as compared to absolute control (73.0 cm). These treatments are also superior to increase the grain yield/plant and 100 grain wt.

Among the various growth hormones, CCC & MH responded well to reduce the plant height in soybean as compared to rest of the growth hormones.

Chemical growth retardants have been used in attempts to decrease vegetative growth during flowering. Particularly in indeterminate varieties so that photo

assimilate could be translocated to flowers and pods rather than vegetative growth. Earlier workers, Greer and Anderson (1965); Hicks et al., (1967). Wax and Pendleton (1968); Bauer et al., (1969) showed that field application of TIBA (2, 3, 5, Triiodobenzoic acid at onset of flowering of indeterminate soybean caused less lodging, shorter plants, earlier maturity more pods/plant small seeds. According to Tanner and Ahmed (1974) growth regulators like TIBA increased yield when growth conditions were favorable, but not when they were poor. Thus results of TIBA reported by earlier worker are similar to the results of GA, MH, CCC, AA in present studies.

References

Baur, M.E., T.C. Sherbeck, and A.J. Ohlrogge. 1969. Agron. J. 61:604-606.

Greer, A.H.L. and I.C. Anderson. 1965. Crop Sci. 5:229-332.

Hicks, D.R., J.W. Pendleton, and W.O. Scott. 1967. Crop Sci. 397-398.

Tanner, J.W. and S. Ahmed. 1974. Crop Sci. 14:371-374.

Wax, L.M. and J.W. Pendleton. 1968. Agron. J. 60:425-427.

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Heritability and genetic advance for polygenic traits in M_2 generation of soybean

Differences in mean values of traits treated and untreated populations were also noticed. Heritability (h_2) and genetic advance (GA) were studied in M_2 progenies for four varieties Monetta, MACS-346, 57 and 124 irradiated to five doses 10, 20, 30, 40, and 50 kR doses. The estimates of heritability in broad sense were higher than control for traits viz., days to maturity (except 30 kR); pods/plant except (50 kR); grain yield/plant and 100 grain weight (at 10 and 20 kR only). Genetic advance as percentage of mean was higher than control for traits viz., plant height, pods/plant and grains/pod. While it was higher in only certain doses for traits branches/plant (40 and 50 kR) and 100 grain weight (10 and 20 kR).

Soybean, a universal crop for better global health which encompasses the concept of soybean as a crop grown worldwide for its value as a source of nutritious food for people and livestock, and as raw material for industrial product, while being at the same time an environmentally friendly renewable resource with an important role in sustainable agriculture.

With a view to generate an information on systematic mutation studies and to utilize the mutants for crop improvement attempt was made in present studies to assess genetic variability through gamma irradiation in M_2 generation of four varieties of soybean treated with five doses i.e. @ 10, 20, 30, 40, and 50 kR and were compared with their control parents.

Material and Methods

M_2 progenies of four varieties viz. MACS-346, MACS-57, MACS-124 and Monetta treated with 10, 20, 30, 40, and 50 kR gamma rays doses were studied for heritability and genetic advance. The seeds of the individual plant were sown as progeny rows adopting spacing of 45 x 10 cm in RBD with two replications.

Observations were made on different characters (Table 1). Heritability in broad sense was estimated as per the method outlined by lush (1940) and genetic advance as per Johnson et al. (1955) and presented in Table 1.

Table 1. Mean performance, heritability and genetic advacne estimated in four gamma irradiated varieties with five doses of gamma rays.

Variety	Gamma Ray Dose (kR)	Days to 50% flowering			Days to Maturity			Plant height (cm)			Pods/plant			Branches/Plant			100 grain weight (gm)			Grain yield/Plant (gm)		
		Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.
MACS-346	0	52.50	99.02	3.91	100.00	86.238	3.89	47.48	98.59	33.64	85.70	94.85	11.80	5.62	79.91	43.34	12.55	72.91	15.98	19.44	95.32	47.98
	10	60.25	94.00	2.51	102.89	62.125	2.34	26.11	94.14	24.92	61.50	99.67	78.02	4.50	53.77	21.41	11.50	90.61	38.39	16.01	97.528	78.10
	20	57.76	76.75	5.86	102.06	88.749	6.85	33.50	96.86	27.19	77.29	99.53	45.18	4.66	19.61	4.38	12.08	77.89	17.07	14.98	97.26	68.41
	30	52.04	83.80	7.69	102.88	93.08	8.69	32.50	97.83	12.40	52.16	99.54	55.352	4.16	25.978	7.91	11.64	50.95	7.17	14.18	97.32	75.50
	40	57.55	88.92	5.00	102.72	89.92	4.89	32.12	96.80	37.50	73.75	99.65	70.64	4.53	38.27	14.00	12.07	50.22	8.22	16.73	88.19	26.44
	50	57.15	79.86	6.00	106.00	87.85	6.15	25.19	84.52	18.529	59.73	69.98	40.25	3.95	-4.71	0.503	12.21	-10.81	1.31	13.48	91.24	44.83
MONETTA	0	41.50	88.66	7.99	84.75	91.55	6.56	40.70	93.62	17.34	79.90	85.71	70.92	3.00	85.56	23.52	12.00	79.91	18.11	7.08	79.16	64.27
	10	49.25	85.01	2.99	113.50	86.04	3.88	33.50	91.60	15.53	62.50	84.68	60.97	3.50	32.73	21.90	10.00	79.00	17.01	6.25	79.16	58.52
	20	47.75	85.11	8.14	101.51	96.96	9.14	31.62	97.69	36.40	55.19	99.42	53.10	4.02	42.73	11.09	12.29	78.13	16.57	11.76	95.56	61.52
	30	47.47	88.49	1.19	99.52	88.38	2.84	33.71	96.74	27.16	49.27	99.62	79.39	4.32	72.35	38.814	12.87	74.65	14.34	11.32	95.18	62.35
	40	40.68	86.77	5.68	105.90	88.66	5.69	24.84	86.35	17.00	49.69	99.34	7.73.53	4.11	52.39	27.18	12.14	-3.45	0.34	11.47	85.42	34.44
	50	40.50	86.91	16.96	95.78	96.99	14.96	31.06	86.37	13.83	61.40	99.57	88.13	4.08	59.03	37.11	11.11	-9.21	0.77	15.46	90.53	41.86

Table 1. continued

Variety	Gamma Ray Dose (kR)	Days to 50% flowering			Days to Maturity			Plant height (cm)			Pods/plant			Branches/Plant			100 grain weight (gm)			Grain yield/Plant (gm)		
		Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.
MACS-57	0	53.01	88.51	19.20	104.25	90.53	18.30	60.25	98.85	30.75	58.85	99.37	60.77	4.06	47.11	19.72	13.00	77.81	16.03	12.17	82.86	33.45
	10	55.50	85.67	5.08	93.38	84.68	4.06	26.49	94.61	32.56	68.54	99.71	87.67	3.68	8.38	13.07	11.51	64.81	16.00	14.93	90.60	41.69
	20	54.33	79.35	3.95	94.02	81.45	2.94	29.56	96.61	32.72	70.67	99.68	66.13	4.15	58.68	25.63	11.87	69.72	13.00	12.90	95.18	61.56
	30	52.05	88.42	6.58	94.29	92.52	5.58	30.72	97.02	29.57	74.12	99.68	55.20	4.40	62.82	25.29	12.12	60.81	8.58	18.13	98.14	70.53
	40	56.88	81.92	5.49	101.00	91.03	4.48	35.75	93.07	28.57	62.15	98.68	53.60	4.14	69.77	24.23	10.27	58.82	9.52	6.17	88.16	68.13
	50	56.90	86.11	6.07	100.95	90.04	5.09	37.80	92.57	19.52	60.00	89.24	52.84	4.00	73.82	23.11	10.95	78.98	8.81	6.95	82.15	66.15
MACS-124	0	55.19	89.98	3.11	106.25	76.98	2.69	66.50	98.03	20.85	28.35	99.29	40.26	2.60	49.33	28.53	12.95	57.85	10.82	17.63	87.96	26.98
	10	56.17	84.68	3.21	102.99	74.68	2.20	29.47	97.09	36.00	69.58	99.72	80.60	4.62	80.99	55.25	10.04	70.52	16.64	13.50	96.20	64.80
	20	52.35	81.64	3.11	103.94	75.99	2.44	25.22	98.31	69.00	72.94	99.50	53.91	4.94	42.66	14.77	10.31	71.90	15.80	16.40	95.78	57.81
	30	53.13	91.04	3.92	101.41	92.08	4.68	36.59	98.56	44.08	77.31	99.60	50.28	5.21	73.91	33.61	11.68	78.61	18.72	15.63	96.28	57.36
	40	57.35	88.11	3.21	102.80	80.77	2.29	33.06	97.00	29.96	77.58	99.76	69.44	5.60	87.45	92.58	10.15	69.12	14.17	13.05	98.36	63.34
	50	58.10	82.12	2.92	103.80	80.89	2.39	39.30	92.50	25.27	64.10	98.88	66.33	3.90	86.45	88.87	10.20	68.11	13.22	6.93	96.39	69.39

Results

The data presented in Table 2 indicated that the means of all the quantitative characters shifted towards either positive or negative directions. The estimates of heritability in broad sense were higher than in the control (kR) for some of the traits viz., day to maturity (except 30 kR), pods/plant (except 50 kR), grain yield/plant and 100 train weight (10 and 20 kR only). While rest of the characters showed lower estimates of heritability than their control.

Table 2. Mean performance, mean heritability (h) and genetic advance (GA) as influenced by different doses of gamma rays and varieties.

Treatments	Days to 50% flowering			Days to Maturity			Plant height (cm)			Pods/plant			Branches/Plant			100 grain weight (gm)			Grain yield/Plant (gm)		
	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.	Mean	h^2	G.A.
A) Gamma Doses (kR)																					
0	51.80	91.14	8.55	98.25	86.28	5.36	97.27	53.83	25.64	3.82	65.48	28.75	63.20	94.81	30.18	14.08	88.26	43.17	12.63	72.12	15.24
10	54.97	87.34	3.44	100.00	76.88	3.12	94.36	28.89	27.20	4.08	43.97	27.91	65.53	95.95	76.81	12.67	96.06	60.77	10.76	76.24	21.90
20	52.85	80.58	5.26	99.48	88.55	5.34	97.36	29.98	41.35	4.44	40.92	13.96	69.02	99.53	54.58	14.01	96.59	62.33	11.42	74.26	23.94
30	51.17	87.93	4.40	101.90	91.52	5.17	97.53	33.38	28.30	4.52	58.76	26.41	63.21	99.61	60.06	14.81	91.80	66.43	11.89	47.95	8.93
40	57.26	86.42	4.85	103.63	85.60	4.61	93.31	31.44	28.25	4.74	61.97	39.42	65.79	99.35	66.80	11.85	91.31	48.08	11.16	45.40	6.44
50	53.16	83.15	7.98	103.19	88.94	4.64	89.07	33.33	19.30	3.98	56.00	37.40	61.30	89.42	61.88	10.71	88.36	55.56	11.11	41.77	6.02
B) Barieties																					
MACS-346	56.21	87.06	5.16	102.76	84.66	5.46	95.42	34.34	25.71	4.35	37.04	15.25	68.36	93.87	50.20	15.80	94.48	56.21	12.00	61.90	15.17
Monetta	44.52	86.82	7.15	80.16	91.43	7.17	92.06	32.57	23.44	4.02	57.47	26.59	59.66	94.72	71.00	10.79	88.07	48.69	11.90	53.70	10.84
MACS-57	54.77	84.99	7.72	97.98	88.38	5.08	95.46	36.76	28.95	3.82	53.43	21.84	65.72	97.33	62.70	11.88	89.52	55.89	12.61	65.16	11.12
MACS-124	55.38	86.26	3.24	103.53	80.23	2.78	96.51	38.35	37.54	4.47	70.13	52.26	64.98	99.46	60.13	13.85	95.16	56.61	12.48	71.65	15.71

As regards genetic advance as percentage of mean, it was lower than control in all the doses for the traits Days to 50 % flowering and maturity while it was higher than control for all the five doses for the traits viz., plant height, pods/plant and grains/pod while higher genetic advance of registered in specific dose treatments like 40 and 50 kR for branches/plant and 100 grain weight 10, 20 kR. This indicated that the fixable genetic components of variance increased in irradiated treatment over control. Thus selections from these dose treatments are likely bring about improvement in yield components. Observation under reports of conformation with results of cowpea reported by Murugan and Subramanian, 1993.

Differences between mean values of quantitative traits in the treated and untreated populations decrease in subsequent generations (Scossiroli, 1965). Negative shift in the mean of seed yield was reported by Mahishi (1986) and Murugan and Subramanian (1993) in M_2 generation of cowpea.

References

Johnson, H.W., Robinson, and R.E. Comstock. 1955. Estimation of genetic variability and environmental variability in soybean. Agron. J. 47:314-318.

Lush, J.L. 1940. Intra size correlation and regression offspring on dams as a method of estimating heritability in characters. Proc. Amer. Soc. Anim. Prod. 33:293-301.

Murugan, S. and M. Subramanian. 1993. Variability studies for polygenic traits in M_3 and M_4 generations of cowpea Vigna unquiculata (L.) Crop Res. 6(2):264-269.

Mahishi, D.M. 1986. Comparison of nature, magnitude and breeding value of polygenic variability induced in homozygous and heterozygous background in cowpea Vigna unquiculata (L.) Walp. Mysore J. Agric. Sci. 18:154.

Panse, V.G. and P.V. Sukhatme. 1957. Statistical methods for agricultural workers. 2nd Ed. ICAR, New Delhi.

Scossiroli, R.E. 1965. Value of induced mutations for quantitative characters in plant breeding. The use of induced mutations in plant breeding. Rad. Bot. 5(supple.):443-450.

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Application of DNA fingerprint to genetic analysis of photo period sensitivity in soybean (Glycine max). (1) Polymorphism of micro satellite DNA in varieties and their hybrids

Photo period sensitivity is one of the most important characteristics for determining soybean adaptability and productivity. In order to expanding adaptable region and increasing grain yield, it is absolutely necessary to ascertain the inheritable manner of photo period sensitivity. For that purpose, it will be very helpful to identify DNA markers linked with this characteristic.

Although several genes controlling flowering date and maturity in soybean have been reported (Bernard et al., 1971; Saindon et al.. 1989), the inheritance of photo periodism in soybean has not been analyzed so precisely, and no genetic markers linked with this trait have been found hitherto.

Eukaryotic genomes are characterized by the occurrence of large amounts of repetitive DNA sequences (Epplen et al., 1988; Tautz et al.. 1989). The simplest repetitive sequences constituted from short (2-4 bp) motives named as micro satellite revealed a surprisingly high degree of variability in man, animal and plant compared with RFLP and mini satellite markers, due to the abundant variability in the number of tandem repeats (Jeffreys et al.. 1985; Hayashi et al.. 1993). These features, together with the genetic manner of co-dominant Mendelian, make them the ideal sources of informative genetic markers to identify interesting genotypes, varieties, individuals and to determine the degree of variability in a population (Weber, 1990).

This paper demonstrates the occurrence of highly polymorphic DNA regions of micro satellites in different varieties and their hybrids of soybean. Varieties coming from various latitudes with different photo period sensitivity had significant differences in motif of micro satellites and the polymorphism of micro satellites had relative stability

among individuals of F_1 hybrids in each cross combination. It was proved that DNA-fingerprinting mediated by simple repetitive oligonucleotide was sufficient to allow for hybrid identification.

Material and Methods

Three F_1 populations were constructed from the crosses between Heihe 5 X Akisengoku, Heihe 5 X No. 29 and Akisengoku X No. 29. The three parents are insensitive, moderately sensitive and highly sensitive to photo period, respectively. The varieties and their F_1 individuals were potted seven days after seeding and grown in a phytotron under the temperature of 280C and natural light. The trifoliate leave were then harvested as the source of DNA extraction.

Plant DNA was extracted from 2.5 g leaf by the modified CTAB method (Saghai-Maroof et al., 1984), and digested with three kinds of restriction enzymes HinfI, HaeIII and Rsa I. Digested DNA was electrophoresed in 1.1% agarose gel and the gel was southern-blotted onto a Hybond TMN membrane (Amersham). The hybridization was carried out with the synthetic oligonucleotide probe (AAT) 6 which was confirmed to be more polymorphic in soybean with strong signals (M.Hayashi et al.. 1993).The probe was ending-labeled using DIG Oligonucleotide 3'-End Labeling Kit (Boehringer Mannheim). Membrane was washed in 2XSSC/0. I % SDS twice at room temperature for 5 minutes and twice at hybridization temperature for 15 min. Detection was conducted according to DIG luminescent detection method (Boehringer Mannheim).

Polymorphism of DNA fragments was evaluated according to the calculation performed by Wetton et al.. (1987). BS = 2NAB/ (NA+ NB) where BS is the level of band-sharing between lane A and B; NAB is number of bands shared by lane A and B; NA and NB are the total number of bands in lane A and lane B, respectively. BS was only calculated between variety genotypes.

Results and Discussion

In order to optimize DNA fingerprinting patterns, DNA extraction from the parent varieties were digested with three kinds of restriction enzymes: Rsa I, HinfI and HaeIII. DNA fragment profiles from the parent genotypes were significantly different with various restriction enzymes (Table 1) The polymorphism of DNA fragments from HinfI

treatment was less than those from other enzymes, and HaeIII enhanced the increment of the number of DNA bands the most effectively. However, BS values of DNA subjected to HaeIII digestion tended to be higher level when compared with the other two enzymes, HaeIII-generated fragments DNA were clustered in the high molecular weight range. Therefore Rsa I-cleaved fragments were found the most suitable for oligonucleotide fingerprinting of soybean since the (AAT) 6-Positive fragments generated by this enzyme had lower BS value and were detected in a size range (i.e. between 0.4 and 7.2 kb), easily analyzable for length polymorphism. From these results it was concluded that the polymorphism detected was partially dependent on the restriction enzymes used.

The existence and distribution of sequences complementary to (AAT) 6 were investigated in three varieties and their F_1 hybrids. Hybridization of (AAT) 6 to the DNA digested by Rsa I resulted in a complex pattern with strong signals covering a wide range of molecular weight and displayed high polymorphism in all the varieties and F_1 individuals (Fig. 1). A distinct inheritance of co-dominance occurred in F_1 hybrids throughout all cross combinations and the number of co-dominant bands was summed to nine (lane 3), twelve (lane 5), and thirteen (lane 6), respectively. Of three combinations, Akisengoku X No. 29 and Heihe 5 X NO. 29 contributed more polymorphic loci. Moreover, the fingerprint of different F_1 individuals derived from a particular combination were identical (data not shown). These results suggested that simple repetitive oligonucleotide probe (AAT)6 was a suitable tool for the unequivocal identification and characterization of varieties and their hybrids with various sensitivity to photo period in soybean.

Table 1. Analysis of DNA fingerprint in three varieties

Enzymes	No. of all bands in varieties			BS value in varieties		
	A	N	H	A/N	H/N	H/A
Rsa I	26	25	20	0.4889	0.4706	0.6086
Hint	22	20		0.6666		
HaeIII	27	31		0.6552		

Note: A=Akisengoku; N=No. 29; H=Heihe 5. (AAT) 6 was used as a probe.

Currently we prove that the segregation ratio of photo-sensitivity in F_2 population seems to be fit to Mendelian 3:1 ratio for cross Heihe 5 X Akisengoku

(unpublished data). In reference to F_2 segregation of micro satellite DNA, a Mendelian 3:1 ratio of (GATA). fragment was found in tomato (Vosman et al., 1992). Furthermore, an attempt is being undertaken to determine the linkage between micro satellite DNA and photo period sensitivity trait using different probes and restriction enzymes.

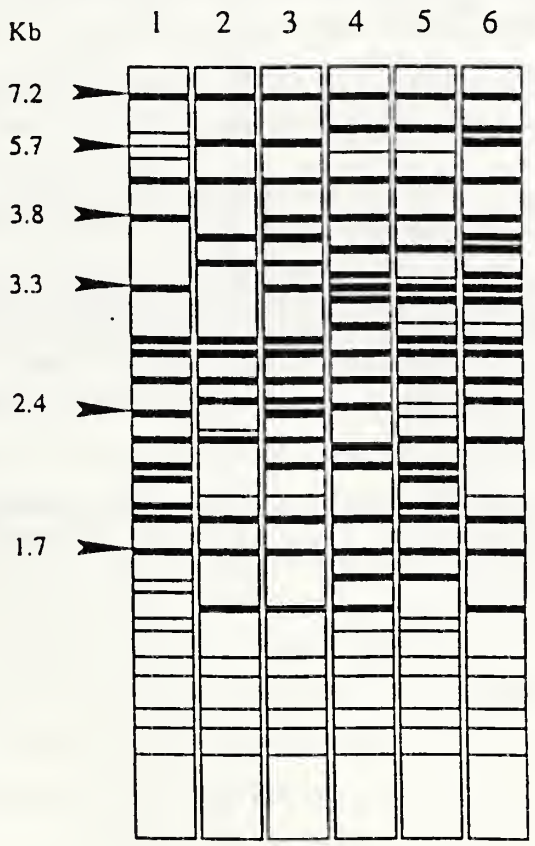

Fig 1. DNA fingerprint patterns of three varieties and their hybrids. DNA was digested with Rsa I and hybridized with (AAT)6. Lane number I through 6 correspond to Akisengoku, Heihe 5, Heihe 5 X Akisengoku, No. 29, Akisengoku X No. 29 and Heihe 5 X No. 29, respectively.

References

Bernard R.L. 1971. Two major genes for time of flowering and maturity in soybean. Crop Science 11:242-245.

Epplen J.T. 1988. On simple repetitive GATA/GACA sequences: a critical reappraisal. J Hered 79:409-417.

Jeffreys A.J., V. Wilson, and S.L. Thein. 1985. Individual-specific "fingerprints" of human DNA. Nature 316:76-79.

Hayashi M., T. Yanagisawa, K. Harada, and T. Akihama. 1993. Simple repetitive DNA sequences in the soybean (Glycine max) genome. Japan J. Breed. 43:449-453.

Rogstad S.H., J.C. Patton, and B.A. Schaal. 1988. M13 repeat probe detects DNA mini satellite-like sequences in gymnosperms and angiosperms. Proc. Natl. Acad.Sci. USA 85:9176-9178.

Saindon G., H.D. Voldeng, W.D. Beversdorf, and R.I. Buzzell. 1989. Genetic control of daylength response in soybean. Crop Science 29:1436-1439.

Tautz D. 1989. Hypervariabality of simple sequences as a general source for polymorphic DNA markers. Nucleic Acida Rea. 17:6463-6471.

Vosman B., P. Arens, W. Rus-Kortekaas, and M.J.M. Smulders. 1992. Identification of highly polymorphic DNA region in tomato. Theor Appl Genet 85:239-244.

Weber J.L. 1990. Informativeness of human (dC-dA)n (dG-dT)n polymorphism. Genomics 7:524-530.

Wetton J.H., R.E. Carter, D.T. Parkin, and D. Walters. 1987. Demographic study of a wild house sparrow population by DNA fingerprint. Nature 327:147-149.

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Population genetic diversity of wild soybeans on the Kanto plain, Japan

Wild soybeans (Glycine soja Sieb. and Zucc.) are common throughout Japan as far north as latitude 42° 40' N, Hokkaido. To better understand the diversity of various wild species, with the objective of determining in situ conservation strategies, we have applied PCR methodology. Randomly amplified polymorphic DNA markers (RAPDs) permit analysis of small scale variation in populations so these markers were used to determine inter- and intra-population diversity of Glycine soja.

Ten populations within a 20 km radius of Tsukuba(140° 5' E, 36° 2' N), 50km northeast of Tokyo, were sampled. All populations were quite small 1ha or less, and mainly in disturbed habitats. Seeds from 10 individuals per population were sown in a greenhouse and leaves sampled when plants had reached the 15-20 leaf stage. DNA was extracted using standard protocol for leaf tissue of between 0.5g and 1g (Williams et al.,1991). Extracted DNA was standardised to 5ng/ul. RAPDs were generated using standard protocol (Williams et al.,1991) on a Techne PHC-3 thermocyler and visualized on starch gels stained with ethidium bromide.

15 polymorphic markers generated from 10 selected primers were scored. We detected polymorphism in 8 out of the 10 populations. Between 2 and 8 banding patterns were detected in the 8 populations showing RAPD marker polymorphism. The average number of banding patterns in each of the 10 populations was 3.7. Although there was some correlation between population location and inter-population diversity two nearby populations showed a high degree of divergence for RAPD markers.

These results confirm the relatively high degree of local variation for Glycine soja on the Kanto Plain (Shimamoto, 1994). Populations of species, from disturbed habitats, which produce abundant seeds that can easily be transported can result in a mosaic of variation. Around Tsukuba, a developing community, wild soybean populations can easily develop or be destroyed, we found a mosaic of variation rather than small scale clinal variation based on RAPD marker polymorphism.

Further studies to determine whether RAPD banding patterns detected

represent loci are underway. The diversity of these populations compared to accessions from the same area stored in the national gene bank of Japan at NIAR is to be investigated.

References

Shimamoto, Y. 1994. Soybean cultivar establishment, development and cellular biology. Research results of a science research grant. Research subject no. 03454033. Agricultural School of Hokkaido University.

Williams, J.G.K., M.K. Hanafey, J.A. Rafalski and S.V. Tingey. 1991. Genetic analysis using randomly amplified polymorphic DNA markers. Methods in Enzymology. 218:704-704.

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Characteristics of 11S protein molecules composed of different intermediary subunits in soybean

The 11S globulin (glycinin) is one of the major soybean storage proteins, accounting for about 35% of the total seed protein. This protein is composed of six nonidentical intermediary subunits, each consists of an acidic polypeptide component (A) linked to basic polypeptide component (B) by a single disulfide bond. Five major subunits have been purified and separated into two groups based on sequence homologies. One is an intermediary subunit group I ($A_{1a}B_2$, $A_{1b}B_{1b}$ and A_2B_{1a}) and the other is group II (A_3B_4 and $A_5A_4B_3$). The sequence homology between the two groups has been shown to be only 60-70% (Nielsen, 1985). We have classified the group II subunit into two subgroups, subgroup IIa ($A_5A_4B_3$ subunit) and IIb (A_3B_4 subunit) because the joint segregation for presence and absence of the 11S intermediary subunits fitted well to the theory ratio for independent inheritance among the three loci controlling the group I, $A_5A_4B_3$ and A_3B_4 subunit,, respectively (Yagasaki et al., in press).

Close relationships between the subunit composition of IIs globulin and the properties of tofu (soybean curd)-gel formation have been reported. The tofu-gel from crude 11S is much harder than that from crude 7S (Saio et al., 1969), which is another major storage protein in soybean seed. Nakamura et al., (1985) indicated that intermediary subunits of 11S played an important role in increasing hardness of heat induced protein gel. Thus, it is necessary to evaluate soybean cultivars having various 11S/7S ratios as well as different subunit compositions of the 11S globulin for processing suitability.

To evaluate properly tofu processing quality of soybeans having different subunit compositions, isogenic lines with the different 11S subunits are being developed.

Material and Methods

F_2 seeds were obtained from the cross between cv. Tamahomare having both the group I and subgroup IIb component and a breeding line having only the subgroup IIa component.

A total globulin fraction was prepared from cv. Fukuyutaka and the respective F_2 seeds as follows: five seeds were powdered and 15 mg of soybean meal were homogenized with 6 ml of 0.05 M tris hydrochloric acid (Tris-HCl) (pH 8.0) globulins were precipitated by adding 180 µl of 1.0 M sodium acetate buffer (pH 4.5) to supernatant (1.8 ml) obtained by centrifugation (15,000 rpm, 3 minutes) of the homogenate. The total globulin fraction was dissolved in 2.0 ml of buffer solution for gel filtration.

Gel filtration of the crude 11S globulin fraction was conducted on a gel-filtration column of Sephacel S-300 (2.3 x 90 cm) using a buffer solution containing 0.05M Tris-HCl (pH 8.0), 0.5 M sodium chloride and 0.049 % sodium bisulfite. Fraction of 7.5 ml was collected and measured at 280 nm using a spectrophotometer. The content of soluble protein obtained by gel filtration was estimated by the Lowry method using bovine serum albumin as the standard protein. A single major peak containing most of the 11S globulin subunits judged by SDS-PAGE was collected. After adjusting the protein content to 0.1% (w/w) antigenicity of the four types of 11S protein molecules were examined on 1.0% agar plate by the double immunodiffusion method. Thirty pl of anti IIS serum and respective antigens were applied to wells. The anti 11S serum from rabbits was prepared using the purified 11S globulin from Fukuyutaka having all the subunits.

Results and Discussion

To elucidate the characteristics of 11S globulin molecules composed of different intermediary subunits, gel filtration was carried out. The globulin concentrate from each F_2 seed having one subunit group (I, Ira or IIb subunit) gave two major peaks at positions nearly corresponding to those of cv. Fukuyutaka having all the subunit groups (I, IIa and IIb subunits)(Fig. 1). F_2 Seeds having two subunit groups (I and IIa, I and IIb and IIa and IIb) also gave the same elution patterns. Using SDS-PAGE 11S globulins were found to be present in the second peak, but no globulins were present in the first one.

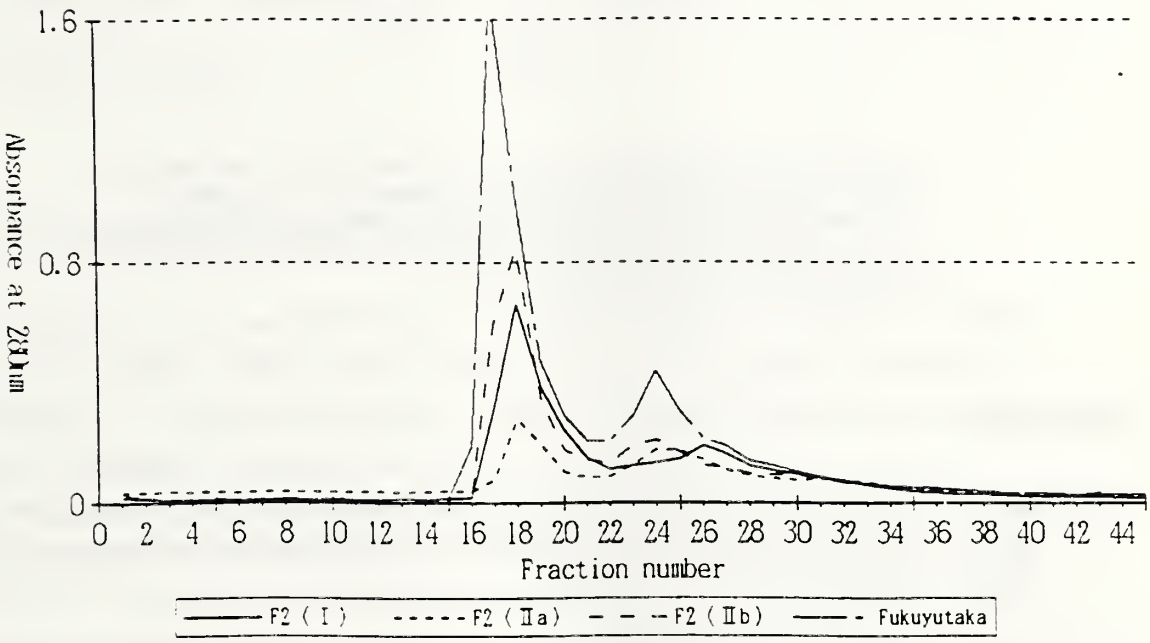

Fig. 1 Gel filtration patterns of the 11S globulins consisted of one subunit group (I, IIa or IIb subunit) on Sephacel S-300 (2.3 x 90cm).

To examine immunological reactivity of the partially purified lis globulins by gel filtration, double immunodiffusion against anti 11S serum was carried out. Globulins reacted with the anti 11S serum to give single precipitin reaction lines. The junetion between precipitin lines were completely fused when F_2 seeds lines, which consisted of group I and subgroup IIa having iis globu. ipitin lines from Fukuyutaka, which has all or I Ib met prec subunits of both groups. However, F_2 seeds having only the group I subunit had a slightly different reaction at this junction with Fukuyutaka, they did not quite fuse. F_2 seeds lacking the group I subunit completely, showed only partial lines from Fukuyutaka. A schematic diagram of this is shown (Fig. 2).

These results suggest that although each intermediary subunit can assemble to form a 11S like protein, there are great immunological differences between the 11S protein having the group I subunit and those lacking this subunit.

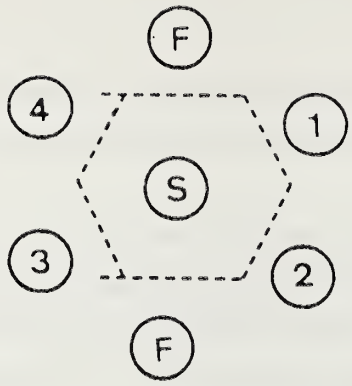

Fig. 2 Schematic diagram of double immunodiffusion on the agar plate containing anti 11S globulin serum (S) against 0.1% 11S globulin solutions obtained from the second peak in gel filtration. (F) Fukuyutaka; (1-2) F_2 seeds having the group I and subgroup IIa (1) or IIb (2); (3-4) F_2 seeds having the subgroup IIa (3) or IIb (4).

References

Nakamura, T., S. Utsumi and T. Mori. 1985. Formation of pseudoglycinins from intermediary subunits of glycinin and their gel properties and network structure. Agric. Biol. Chem. 499:2733-2740.

Nielsen, N.C. 1985. The structure and complexity of the 11S polypeptide in soybean. J. Am. Oil Chem. 62(12):1680-1686.

Saio, K., M. Kamiya, and T. Watanabe. 1969. Food processing of soybean 11S and 7S proteins. Part I. Effect of difference of protein components among soybean varieties on formation of tofugel. Agric.Biol.Chem. 33(9):1304-1308.

Yagasaki, K., N. Kaizuma and K. kitamura. Inheritance of 11S globulin subunits and characterization of 11S protein molecules lacking the subunits in soybean [Glycine max (L.) Merr.] . Japan. J. Breed. in press).

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Evaluation of induced chlorophyll-deficient mutants of soybean

Major efforts have been made to isolate mutants chemically or through mutagenic radiation which could be useful in studies on genetics, breeding, physiology, and biochemical research. When using the mutation breeding technique, one should obtain chlorophyll-deficient mutants easily.

Benedict (1972) reported that the chlorophyll deficiency in most of these mutants could lack the well defined grana structure of the chloroplasts. A possible mechanism to explain the chlorophyll abnormalities has been suggested on the basis of the decrease in malate dehydrogenase (MDH) activity (Bidlack et al., 1991; Elmer et al.,1994). Recently it was reported that conditional lethality involved a cytoplasmic mutant and chlorophyll-deficient malate dehydrogenase mutants in soybean (Palmer, 1992; Minor and Palmer, 1994). These conditional lethal genotypes were suggested to be unique biological materials to characterize the interaction between organelle and nuclear genomes.

In M_2 generation after mutagenesis of soybean seeds with ethyl methanesulfonate (EMS), we selected several types of chlorophyll-deficient mutant lines (Lee et al., 1994). The aims of this research were to verify the inheritance of these characters in M_3 generation as well as to compare the chlorophyll composition of mutants with wild-type soybean plants.

Materials and Methods

Seeds of two recommended soybean varieties in Korea, 'Hwanggeumkong'

and 'Baekunkong', were mutagenized with 50mM EMS for 6 hrs at room temperature. Mutagenized seeds were planted directly in the field after postwashing. M_1 plants were harvested individually to give M_2 families next year.

Segregation of chlorophyll-deficient characters were evaluated in M_2 and M_3 generation. Goodness of fit between observed and expected segregating ratio of normal and chlorophyll deficient mutants was evaluated using chi-square analysis.

For determination of chlorophyll concentration, the leaf samples were taken at V_6 stage in the third node from the top of soybean plants grown in the greenhouse. Leaf disks were placed in 80% (V/V) acetone for 7 days at 4 °C in darkness. Absorbances were measured at 657 nm and 664 nm with spectrophotometer.

Results and Discussion

Several mutants in growth characters including leaf chlorophyll content were present in M_2 generation. Of 496 M_2 families, 11 families showed chlorophyll-deficient characters. Chlorophyll-deficient mutants could be distinguished by several types such as light green color, albino in leaf margin, and light green color outside leaf veins. However, each type of chlorophyll-deficiency were consistent within the line.

If chlorophyll deficiency was governed by single recessive gene (as evidenced indirectly from the data on the segregation of normal to mutant of M_3 generation in Table 1) and all cells in an M_1 seed were mutated completely, segregation ratio should be fit to the ratio of 3 (normal) 1 (chlorophyll-deficient). However, as shown in Table 2, most of all M_2 families except MD69 didn't follow the expected ratio, suggesting that EMS-induced mutation occurred partly, that is, mutated M_1 seed represented chimera.

The M_2 plants, which were thought to be heterozygous for chlorophyll-deficient mutants, segregated into normal to mutant in M_3 generation (Table 1). The observed ratio of normal to chlorophyll-deficient plants in each segregated M_3 line indicated a good fit to the expected ratios of 3 (normal) to 1 (chlorophyll-deficient). Nonsignificant chi-square test of heterogeneity indicated that the data from M_3 line could be pooled. The pooled M_3 data also indicated a good fit to the expected ratios of 3:1. From these results, it could be surmised that single recessive gene confers chlorophyll-deficient characters. Further genetic crosses should be made to confirm precisely the mode of inheritance of these characters.

Table 1. Segregating ratios of chlorophyll-deficient mutants in mutants in M_2 generation.

Wild-type	M_2 line	Observed			$\chi^2(3:1)$	P
		Normal	Mutant	Total		
Hwanggeumkong	MB88	74	1	75	22.40	<0.005
	MB98	77	2	79	21.27	<0.005
Baekunkong	MD69	30	8	38	0.32	0.50~0.70
	MD75	60	6	66	8.91	<0.005

Table 2. Chi-square (χ^2) goodness of fit values for the M_3 line showing the chlorophyll-deficient mutants.

M_3 line	Observed			$\chi^2(3:1)$	P
	Normal	Mutant	Total		
MB88	78	24	102	0.12	0.50-0.75
MB98	60	23	83	0.33	0.50-0.75
MD69	48	14	62	0.19	0.50-0.75
MD75	60	16	76	0.63	0.25-0.50
Total	246	77	323		
$X_T^2(df=1)^*$				0.23	0.50-0.75
$X_d^2(df=2)^{**}$				1.04	0.75-0.90

*,** Deviation and heterogeneity chi-square, respectively.

The wild-type Baekunkong had higher leaf chlorophyll content than light green mutant, as was expected by the appearances (Table 3). Spectrophotometric determination gave a significant genotypic difference in chlorophyll a/b ratio which was 2.85 for normal plant, and 2.95 and 2.27 for MD69 and MD75, respectively. It was interesting to note that there was no difference in chlorophyll a/b ratio between Baekunkong and MD69. However, MD 69 showed the lowest ratio of chlorophyll a/b,

indicating that genotypic difference in the relative reduction in chlorophyll a and b should not follow the same trends.

Table 3. Chlorophyll concentration of leaves of normal and light green mutants of Baekunkong.

	Normal	Mutant	
	Baekunkong	MD69	MD75
	gm^{-2} leaf area		
Chlorophyll a	17.5[a*]	12.3[b]	9.4[c]
Chlorophyll b	6.1[a]	4.2[b]	4.1[c]
Total chlorophyll	23.7[a]	16.4[b]	13.5[c]
	Ratio		
Chlorophyll a/b	2.85[a]	2.95[a]	2.27[b]

* Within row, means not followed by the same letter are significantly different at $P<0.05$ based on LSD.

References:

Benedict, C.R. 1972. Net CO_2 fixation in yellow-green plants. In C.C. Black (ed.), Net Carbon Dioxide Assimilation in Higher Plants. pp. 7-25. Proceedings of a Symposium held at the University of South Alabama, Mobile, Alabama, April 7, 1972.

Bidlack, J.E., L.A. Bidlack, W. Caire, R.G. Palmer, and R.C. Shoemaker. 1991. Subcellular enzyme activities of mutant soybean lines lacking mitochondria MDH isozyme bands. Soybean Genet. Newsl. 18:319-323.

Elmer, A.M., C.A. Barton, and J.E. Bidlack. 1994. Hypothetical mechanism for chlorophyll abnormalities in mutant soybean [Glycine max (L.) Merr.] lines. Soybean Genet. Newsl. 21:199-203.

Lee, H.S., and S.H. Lee, and S.D. Kim. 1994. Evaluation of mutagenesis altering soybean nodulation with ethyl methanesulfonate. WSRC V. (in press).

Minor, V.C. and R.G. Palmer. 1994. Implications of a nuclear inherited chlorophyll retention mutant($d_1d_1d_2d_2$) on conditional lethality in soybean [Glycine max (L.) Merr.]. Soybean Genet. Newsl. 21:207-209.

Palmer, R.G. 1992. Conditional lethality involving a cytoplasmic mutant and

chlorophyll-deficient malate dehydrogenase mutants in soybean. Theor. Appl. Genet. 85:389-393.

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Correlation between average temperature during phenological phases and some morphological characteristics of flowering and efficiency of flowering

Soybean is a short day plant. It needs high temperature for good yielding. Polish climate is unfavorable for soybean, the days are long and temperature is low during flowering and pod setting period. The average daily temperature in June is 16.6 °C and in July is 18.1 °C. The effect of temperature during these periods was studied by many authors. Thomas and Raper (1978) reported that combination of 26/22 °C day/night temperatures was the best for generative development. Enken (1959) found that 17-18 °C was minimal temperature for flowering period. Schmid and Keller (1980), Hume and Jackson (1981), Soldati et al., (1983) showed that low temperature affected negatively pod setting. However, there is only a little information about correlation between temperature during these periods and efficiency of flowering. Szyrmer and Janicka (1985) found positive correlation between the sum of minimal temperatures during flowering period and number of flowers. Kahnt and Hijazi (1987) reported positive correlation between temperature during vegetation period and yield. Positive correlation between minimal daily temperature and yield in Poland was found by Lykowski (1984).

Materials and Methods

38 soybean genotypes were studied. Three old Polish, one from former SU and one Swedish cultivar (Fiskeby V). Moreover 33 soybean plant introductions, within maturity group 00-IV, were obtained from the USDA germplasm collection. The experiments were carried out in 1978-80 on the field of Agricultural Experiment Station at Swadzim near Poznan. The experiments were arranged in complete random block design with three replications. Every plot consisted of three rows 3.0 m long. Five plants from middle row of each plot were randomly selected for precise observations of flowering and pod setting. Temperatures were recorded at the local Meteorological Station at Swadzim. Average temperatures of all periods were calculated separately

for each genotype and, based on gathered data, correlation coefficients were calculated.

Results and Discussion

In the years of experiments the temperatures werevery different, so it was possible to test genotypes in cold (1978), moderate (1980) and warm (1979) weather (Table 1).

Table 1. Average daily temperatures during period from sowing to flowering and flowering period.

Period	Year		
	1978	1979	1980
Average daily temperature from sowing to flowering	13.9	15.9	12.2
Average daily temperature during flowering period	15.6	17.1	16.7
Average daily minimum temperature during flowering	10.2	11.7	12.9

The greatest significant correlations were recorded between average daily temperature during period from sowing to flowering and the first flowering nod number (Table 2), and number of flowers per plant. The correlations with number of clusters and number of flowers on the main stem were significant, too (Table 2). It agrees with the data obtained by Van Schaik and Probst (1958) who found that number of flowers per plant increased with increasing of temperature.

With exception of the correlation between average daily temperature during flowering period and flowering period all correlations presented in Table 3 were significant. The correlation between average daily temperature and period of cluster flowering was distinctively negative. Positive influence of temperature on pod number was found by Tanasch and Gretzmacher (1991).

Table 2. Correlations between average daily temperature during period from sowing to flowering and some characteristics of flowering.

Characteristics	Correlation coefficients
Nod number with first flower	0.86**
No. of clusters per main stem	0.26**
No. of flowers per cluster	-0.16
No. of flowers per main stem	0.19*
No. of flowers per plant	0.52**

*,** Significant at P = 0.05 and P = 0.01

Table 3. Correlations between average daily temperature during flowering period and some characteristics of flowering and efficiency of flowering.

Characteristics	Correlation coefficients
Flowering period	-0.11
Flowering period of cluster	-0.41**
Pods/plant	0.23*
Seeds/plant	0.18*
Percent of pods setting on main stem	0.20*
Percent of empty pods	0.24**

*,** Significant at P = 0.05 and P = 0.01

In correlations between average minimal daily temperature and other characteristics shown in Table 4 only the correlation with period of cluster flowering was significant. No any significant correlation between minimal temperatures and characteristics of reproductive efficiency was found (Table 4). It was opposite to the findings of Lykowski (1984) who stated that minimal temperature influenced yielding more than average daily temperature.

The effect of the average daily temperature during flowering on pod setting is evident if one compares these temperatures with average percent of pod set on plant 15.6 °C - 12.3%, 17.1 °C - 28.9%, 16.7 °C - 18.7%. However, pod setting is affected by

many other factors: draught (Sionit and Kramer, 1977), photoperiod (Tanasch and Gretzmacher, 1991), lack of nitrogen and calcium (Mann and Jaworski, 1970), plant density (Dominguez and Hume, 1978) etc. It is impossible to separate effects of these factors on pod setting in the field experiment. In our experiment the draught was the main factor causing extremely low pod setting in 1978 but it occurred also in 1979 when pod setting was the best. The reaction on long daylength conditions was the main factor of low pod setting in late genotypes.

Table 4. Correlations between average daily minimal temperature during flowering period and some characteristics of flowering and efficiency of flowering.

Characteristics	Correlation coefficients
Flowering period	- 0.14
Flowering period of cluster	- 0.42*
Pods/plant	0.02
Seeds/plant	- 0.02
Percent of pods setting on main stem	0.12
Percent of empty pods	0.17

* Significant at P = 0.01

References

Dominguez, C. and D.J. Hume. 1978. Flowering, abortion, and yield of early-maturing soybeans at three densities. Agron. J. 70:801-805.

Enken, V.B. 1959. Soja. Moskva: 269-280.

Hume, D.J. and A.K.H. Jackson. 1981. Pod formation in soybean at low temperatures. Crop Sci. 21:933-937.

Kahnt, G. and L.A. Hijazi. 1987. Establishment of correlations between environmental factors and yield of soybean cultivars. Eurosoya 6:48-53.

Lykowski, B. 1984. Warunki klimatyczne rozwoju i plonowania soi w Polsce. Rozprawy Naukowe i Monografie. Wydawnictwo SGGW-AR, Warszawa. 1-84.

Mann, J.D. and E.G. Jaworski. 1970. Comparison of stresses which may limit soybean yields. Crop Sci. 10:620-624.

Schmid, J. and E.R. Keller. 1980. The behavior of three cold-tolerant and a standard soybean variety in relation to the level and the duration of a cold stress. Can. J. Pl. Sci. 60:821-829.

Sionit, N. and P.J. Kramer. 1977. Effect of water stress during different stages of growth of soybean. Agron. J. 69:274-277.

Soldati, A., E.R. Keller, H. Brenner, and J. Schmid. 1983. Adaptation of soybeans to northern regions: A review of research work conducted at the Swiss Federal Institute of Technology (1971-1982). Eurosoya 1:27-34.

Szyrmer, J., and M. Janicka. 1985. Screening of soybean genotypes for cold-tolerance during flowering. Eurosoya 3:51-54.

Tanasch, L. and R. Gretzmacher. 1991. Influence of photoperiod and temperature on flower induction of soybean. Eurosoya 7/8:10-15.

Van Schaik, P.H. and A.H. Probst. 1958. Effects of some environmental factors on flower production and reproductive efficiency in soybean. Agron. J. 50:192-197.

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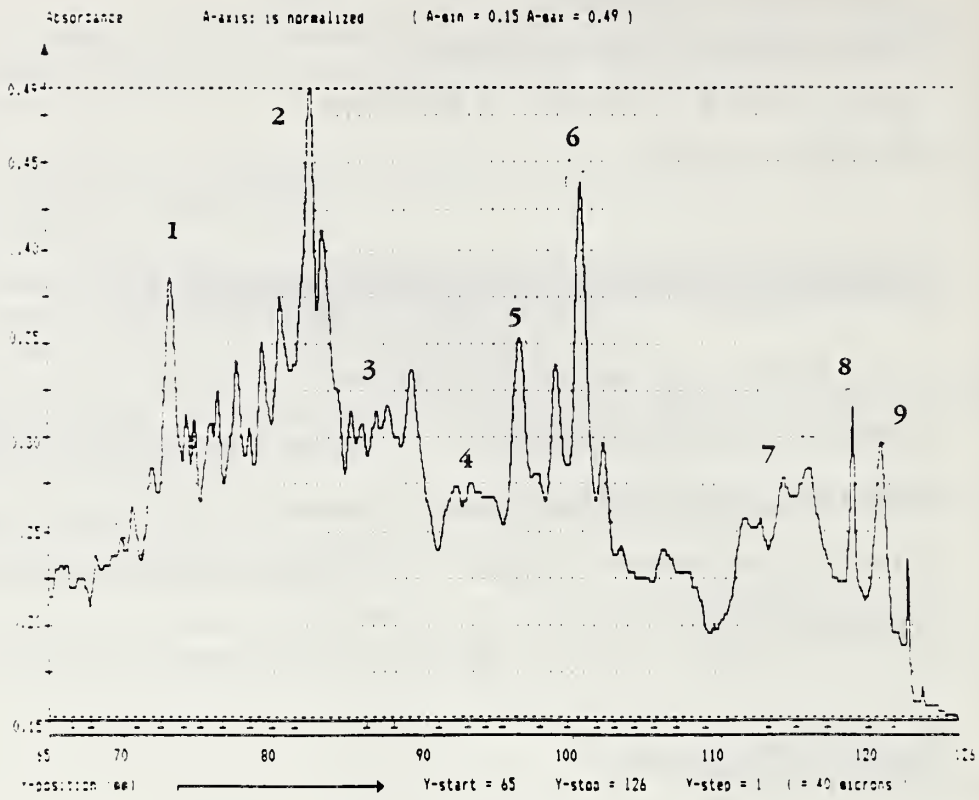
Quantitative analysis of proteins in some soybean mutants

Soybean can be considered as one of the most valuable crops also due to its proteins content and composition. Therefore, there was one of the reasons why we were interested in the investigation of proteins characterization and quantification in some obtained soybean mutants. For this purpose we selected material and methods mentioned below.

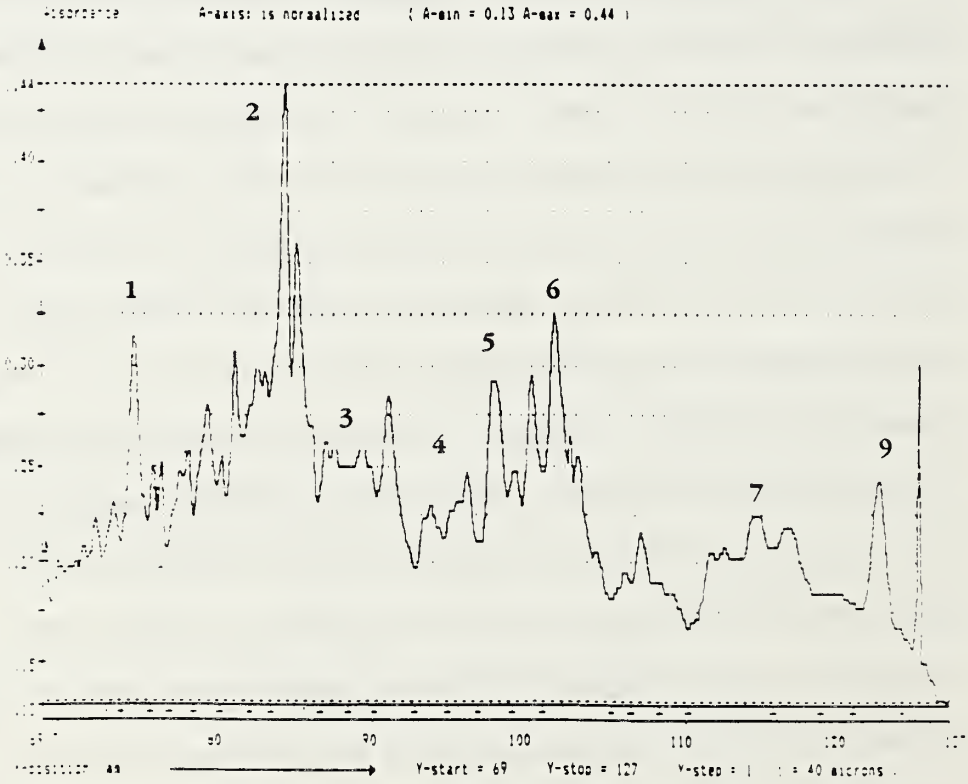
Material and Methods

In our analysis we used 12 mutants that were selected from the variety Nadneprjanskaja after mutagens EI, AS and laser cmission treatment. The variety Nadneprjanskaja was used as the control because the objective of this paper was to observe differences in proteins spectra in mutants originated from mentioned variety. Proteins were extracted according Laemmli (1970) from leaves of 10 days plants which were grown under the temperature of 21 °C in greenhouse. The content of proteins was found according Bradford (1976). On the basis of these results we deternfined equivalent extraction of proteins (10 µg) for each sample. The extractions of proteins were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) on 15% gel and for more detailed deterni-ination of proteins we used the standard Low Range, (M.W. 14200-66000), (Product No. 5630), Sigma. SDS polyacrylamidc gels were stained with Coomassie brilliant blue G-250 and evaluated using densitometer (Ultrascan XLLKB, Bromma, Sweden).

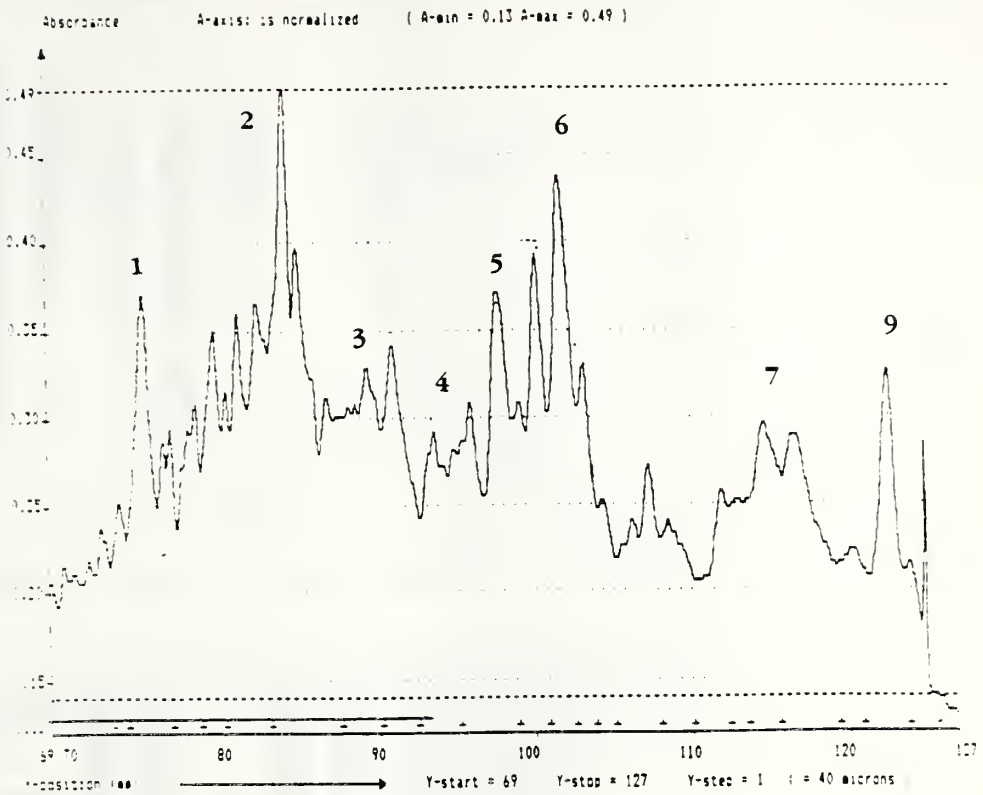
Graph 1 Variety Nadneprjanskaja



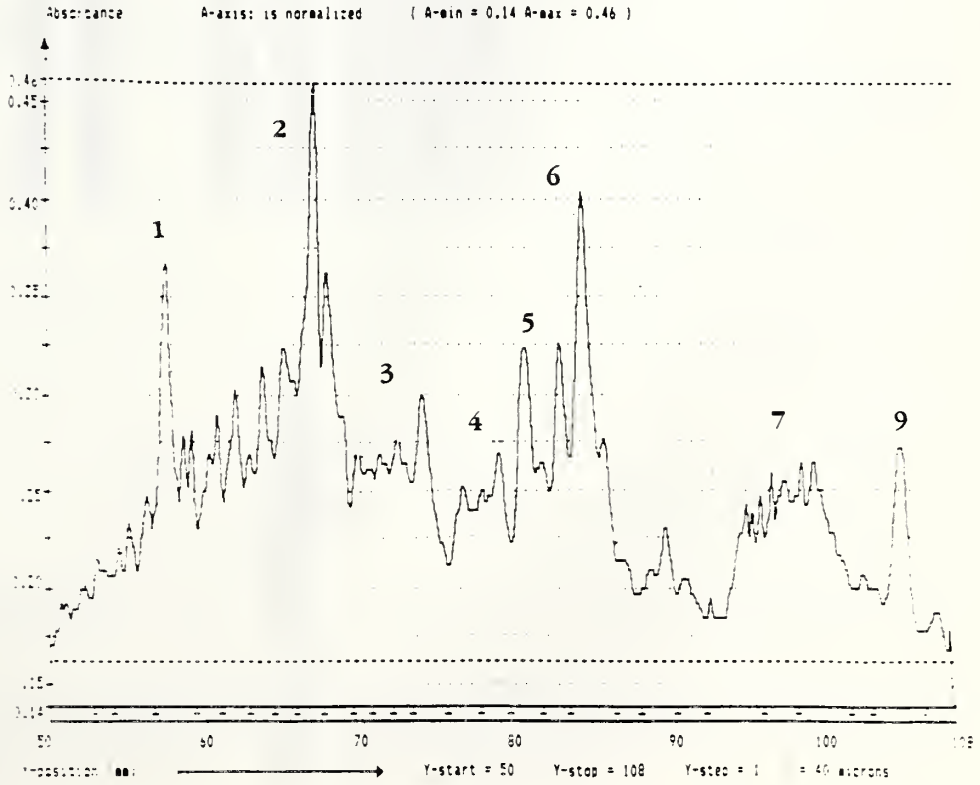
Graph 2 Mutant 3



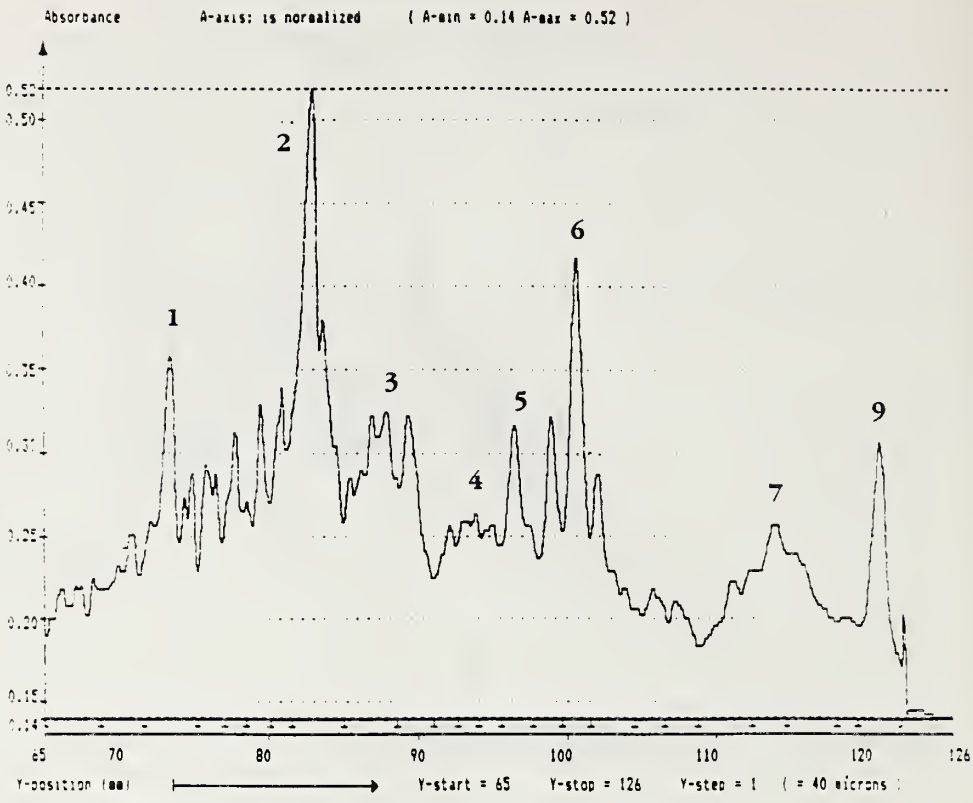
Graph 3 Mutant 4



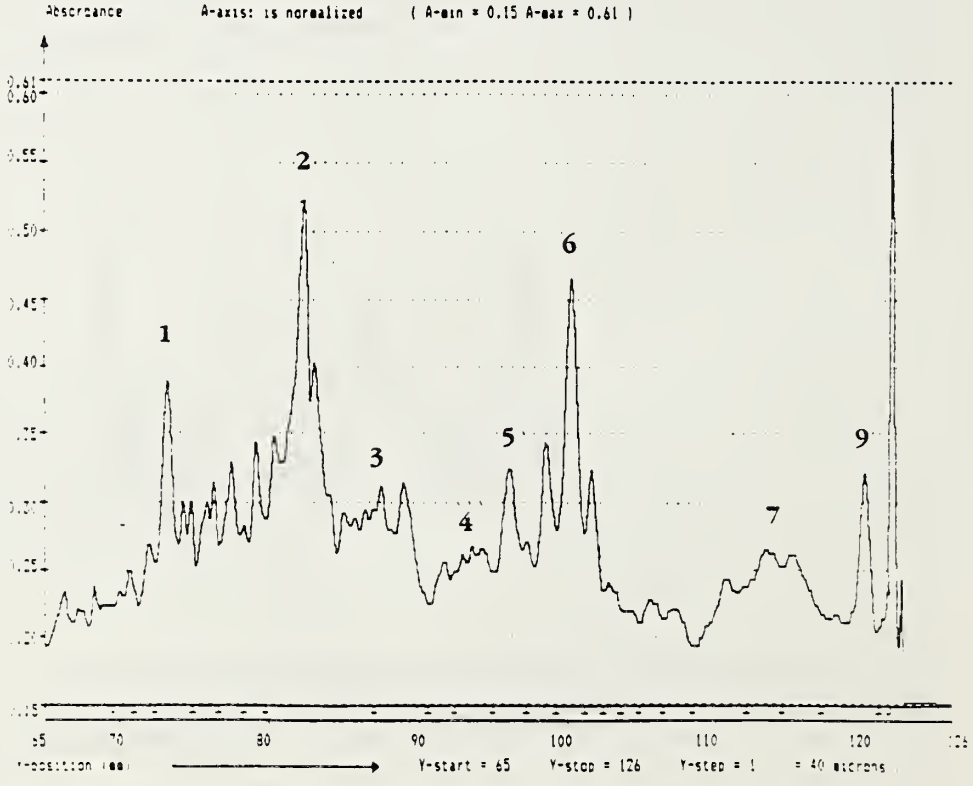
Graph 4 Mutant 7

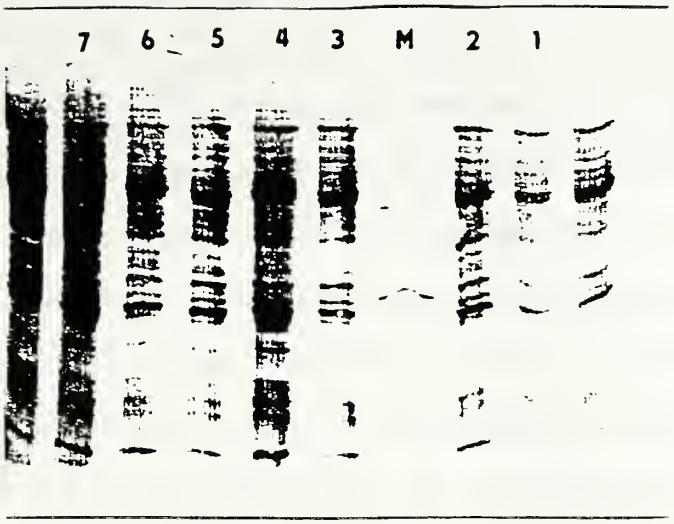


Graph 5 Mutant 9

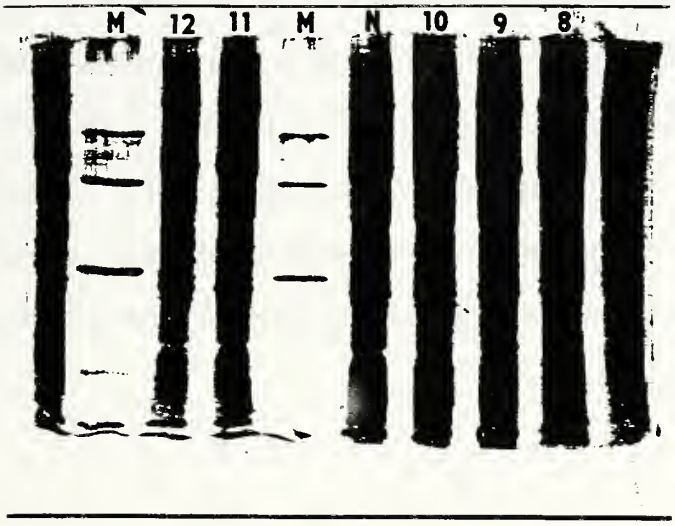


Graph 6 Mutant 11





SDS - PAGE gel of mutants 8 - 12 + Nadneprjanskaja + marker



PS 1 78kD (peak 1)

LSU - Rubisco 53 - 56kD (peak 2)

PS2 46kD (peak 3)

Proteins of the group D (D1-D2) 34kD (peak 4)

LHCP 28kD (peak 5)

PS2 26kD (peak 6)

Precursors of SSU - Rubisco 18 - 21kD (peak 7)

unknown proteins 15 - 16kD (peak 8)

SSU - Rubisco 14.5kD (peak 9)

Results and Discussion

Protein extracts from leaf tissues were subjected to SDS-PAGE as described in Material and methods. The same method of proteins separation and characterization was used in pea and wheat flour in papers of Adamska (1991), Kolster (1992). Honeycut (1989) described major storage protein subunits in association with deficiency of certain aminoacids in normal and shriveled seeds. In the original variety Nadneprjanskaja we observed the protein band at 15-16 Kd while in all of mutants it was missing or was on low level (Graphs 1-6). The mutants 1, 2, 3, 5, 7, 9 have lower expression of proteins SSURubisco, PS1, PS2-46kD, LSU-Rubisco compared to the original variety Nadneprjanskaja. However, higher content of protein subunits SSU-Rubisco, PS2-26kD, PS2-46kD and LSU-Rubisco in the mutants 4, 8, 10, 11 and 12 are shown at graphs 1-6. The mutant 6 has no differences in protein spectra compared to original variety. Klauer (1991) observed proteins at various stages of physiological development in nodulated and nonnodulated plants. The results confirm that 27 and 29 kD proteins of soybean leaf are stored in the paraveinal mesophyll vacuole and show that they are accumulated early during leaf development while they are still strong sinks for nitrogen.

References

Adamska, I., and K. Kloppstech. 1991. Evidence for an association of the early light-inducible protein (ELIP) of pea with photosystem II. Plant Mol.Biol. 16:209-223.

Bradford, M. 1976. A rapid method for the quantitation of rificrogram quantities of protein utilizing. The principle of protein dose binding. Anal. Bioch. 72: 248.

Honeycut, R.J., J.W. Burton, R.G. Palmer, and R.C. Shoemaker. 1989. Association of major seed components with a shriveled-seed trait in soybean. Crop Sci. 29(3):804-809.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Kluauer, S.F., V.R. Franchesci, and M.S.B. Ku. 1991. Protein compositions of mesophyll and paraveinal mesophyll of soybean leaves at various developmental stages. Plant Physiol. 97(4):1306-1316.

Kolster, P., C.F. Krechting, and M.J. GelderW. 1992. Quantification of individual high molecular weight subunits of wheat glutenin using SDS-PAGE and scanning densitometry. J. Cereal Sci. 15:49-61.

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Observation on outcrossing of gamma irradiated 81-1-145 soybean line

Seeds of white-flowered 81-1-145 soybean line (Smutkupt et al., 1984, 1985 and Smutkupt, 1987) were irradiated with 15 krad in the gammater of the Department of Applied Radiation and Isotopes, Kasetsart University and planted in alternate rows with a non-irradiated purple-flowered 81-1-038 line (Smutkupt et al., 1988). As a control experiment, non-irradiated seeds of 81-1-145 were also planted in alternate rows with 81-1-038, which was used as a pollen donating line. The experiments were carried out at the Royal Pang Da Agricultural Station, Amphur Samoeng, Chiang Mai from January to May, 1994.

At the harvesting time, 180 plants of 81-1-145 rows from each experiment were harvested and threshed singly. The seeds of each plant were planted as plant-to-row in the following season from July to November, 1994. At flowering time, individual plant flower color was carefully checked. The purple-flowered plants were tagged.

Among 180 rows of gamma irradiated plants, 132 rows survived. Within these 132 rows, the purple-flowered plants were found in 10 rows. About 7.5 percent of the outcrossing can be estimated.

Among 180 rows of non-gamma irradiated plants (control), 157 rows survived. Within these 157 rows, the purple-flowered plants were observed in 9 rows. About 5.7% of the outcrossing can be estimated. The purple-flowered plants of both 10 and 9 rows were singly and seperately harvested.

In the third generation, the seeds of each purple-flowered plants were planted as plant-to-row in January, 1995 at the Royal Pang Da Agricultural Station, Chiang Mai. The objective of this experiment is to observe a segregation among progenies of the harvested purple-flowered plants.

It is assumed that a purple-flowered plant derived from the gamma irradiated white-flowered parent would be resulted from outcrossing or dominant mutation.

At present, the plants are in the field for further observation. It is expected that two phenonmena; (a) a segregation of 3 purple-and 1 white-flowered plants, and (b)

no segregation of 3:1, could be observed among purple-flowered plants derived from the gamma irradiated parent of 10 rows. The reasons could be an outcrossing for (a) and a dominant mutation for (b), respectively.

Among the purple-flowered plants derived from the non-gamma irradiated parent of 9 rows, there should be only segregating progenies expected, because the white-flowered parent of 81-1-145 was not irradiated.

Should only homozygous purple-flowered plants (no segregation) also be occurred, then a question of unstable gene of flower color (w -> W) in the soybean line 81-1-145 would arise.

References

Smutkupt, S. 1987. Plant gene mutation. 1987. Proceedings of the 5th Genetics Conference of the Genetics Society of Thailandi. Prince of Songkla University, Songkla, Thailand. pp 127-129 (in Thai).

Smutkupt, S., A. Wongpiyasatid, and S. Lamseejan. 1984. New Achievement in research on soybean mutations induced by gamma rays. International Symposium on Genetic Manipulation in Crops. Beijing, China.

Smutkupt, S., A. Wongpiyasatid, and S. Lamseejan. 1985. Observation on back mutation of white-flowered "Wakashima" mutants. Soybean Genetics Newsletter 12:67-69.

Smutkupt. S., A. Wongpiyasatid, and S. Lamseejan. 1988. Improvement of rust resistance in soybean by mutation breeding. Proceedings of a workshop on the improvement of grain legume production using induced mutations. International Atomic Energy Agency, Vienna. pp 371-381.

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Allelic loci in Peking soybean conditioning resistance to Heterodera glycines races 3 & 5

Heterodera glycines Ichinohe (SCN) is the most devastating soybean pest worldwide. Genetically controlled resistance to SCN was first identified in cv. Peking (Ross and Brim, 1957). Peking, a selection from P.I. 17852B, was found to be resistant to SCN Races 1,3, and 5, additionally was also reported to be resistant to Race 6 (Rao-Arelli et al., 1992a). A recent study indicated that resistance in Peking to SCN Race 3 isolate is conditioned by a combination of 3 genes rhg_1, rhg_2, and Rhg_4 (Rao-Arelli et. al., 1992b). These loci in Peking and P.I. 90763 are found to be allelic to SCN Race 3 isolate and the progenies from the crosses involving resistant parents, Peking X P.I. 88788 and P.I. 90763 X P.I. 88788 segregated both in F_2 and F_3 generations (Rao-Arelli and Anand, 1988). Furthermore, P.I. 88788 conditioned resistance to SCN Race 3 isolate with a combination of two dominant and one recessive genes (Rao-Arelli et al., 1992b).

Both Peking and P.I. 90763 are resistant to SCN Race 5, whereas, P.I. 88788 is susceptible. Each of these two resistant lines Peking and P.I. 90763 incrosses with P.I. 88788, segregated into 1 Resistant : 15 Susceptible in both F_2 and F_3 generations indicating two recessive genes rhg, rhg which condition resistance to SCN Race 5 isolate. In this report, data have been presented on the genes in Peking conditioning resistance to SCN Race 5 and their association for resistance to Race 3 in crosses with a highly susceptible cv. Essex.

Materials and Methods

A field population of SCN Race 5 was obtained from the Rhodes Farm of the University of Missouri near Clarkton, Missouri and relatively a homogeneous population was developed (Rao-Arelli and Anand, 1989).

Crosses were made between resistant (R) Peking and susceptible (S) Essex. Ten F_1 plants and 308 F_2 plants were evaluated in the greenhouse for their reaction to SCN Race 5 isolate as described by Rao-Arelli and Anand (1988). A set of differentials consisting of Peking, P.I. 90763, P.I. 88788, Picket-71, P.I. 437654 and a

susceptible Essex were included as standard hosts in evaluation. Each differential consisted of 10 plants. The inoculation techniques were already described (Rao-Arelli et al., 1991).

Thirty days after inoculation, plant roots were washed, and the dislodged white females were counted using a stereomicroscope. Plants were classified as resistant or susceptible based on the reaction of the parents. All the F_2 plants with a similar range of white females i.e. 0-15 that occurred on the resistant parent Peking were included in the resistant category. Chi-Square analysis was used to test goodness of fit to appropriate hypotheses.

Results and Discussions

Means and ranges of white females for parents, standard differentials, F_1's and F_2's of the cross obtained in this report are provided in Table 1. All F_1 plants were susceptible, their means and ranges were below that of susceptible F_2 plants and susceptible parent cv. Essex, suggesting that dominance is partial.

Table 1. Range, mean numbers of white females per plant obtained for F_1's, F_2's (derived from cross Peking (R) X Essex (S), parents and standard differentials to SCN Race 5 isolate in greenhouse.

	Range of white females	Mean	Resistant Plants (number)	Susceptible Plants (number)	Genetic Ratio	χ^2	p Value
Generation							
F_1	68-89	77	---	10	---	---	---
F_2	0-12	8	12	---	---	---	---
F_2	18-198	103	---	296	3 : 61	0.14	>0.90
P_1	1-15	7	10	---	---	---	---
P_2	123-178	160	---	10	---	----	---
Standard Differentials							
PI 90763	0-1	0.6	10	---	---	---	---
PI 88788	74-89	123	---	10	---	---	---
Picket-71	42-97	68	---	10	---	---	---
PI 437654	0-1	0.3	10	---	---	---	---
Peking	1-15	7	10	---	---	---	---
Essex	123-178	160	---	10	---	---	---

The observed resistance vs. susceptible frequencies for F_2 plants are also given in Table 1 for three-gene hypothesis. The F_2 plants segregated very closely to 3R:61S, the expected trihybrid ratio with one dominant and two recessive genes for resistance (Rhg, rhg, rhg). A similar segregation was also reported for the same cross in F_2 generation to SCN Race 3 isolate. Therefore, based on the F_2 data, the possibility that resistance to both SCN Race isolates 3 and 5 conditioned by the same set of genes could not be ruled out. We await F_3 data before we confirm the results.

References

Rao-Arelli, A.P. and S.C. Anand. 1988. Genetic relationships among soybean plant introductions for resistance to race 3 of soybean cyst nematode. Crop Sci. 28:650-652.

Rao-Arelli, A.P. and S.C. Anand. 1989. Genetic analyses of soybean genotypes resistant to soybean cyst nematode race 5. Crop Sci. 29:1181-1184.

Rao-Arelli, A.P., K.W. Matson, and S.C. Anand. 1991. A rapid method for inoculating soybean seedlings with Heterodera glycines. Plant Dis. 75:594-595.

Rao-Arelli, A.P., S.C. Anand, and J.A. Wrather. 1992b. Soybean resistance to soybean cyst nematode race 3 is conditioned by an additional dominant gene. Crop Sci. 32:862-864.

Rao-Arelli, A.P., J.A. Wrather, and S.C. Anand. 1992a. Genetic diversity among isolates of Heterodera glycines and sources of resistance in soybeans. Plant Dis. 76(9):894-896.

Ross, J.P. and C.A. Brim. 1957. Resistance of soybeans to the soybean cyst nematode as determined by a double-row method. Plant Dis. Rep. 41:923-924.

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A modified starch gel electrophoresis procedure for resolving soybean alcohol dehydrogenase band 1

Electrophoresis is commonly used in genetic studies and for line identification in cultivar improvement programs of many crops including soybean. Both starch gel electrophoresis and polyacrylamide/starch gel electrophoresis have been used in earlier studies. While these systems have worked successfully for most enzymes, resolution of alcohol dehydrogenase (ADH EC 1.2.3.4) from soybean has proved problematic. Cardy and Beversdorf (1984) used starch gel electrophoresis and reported that ADH, though present, was poorly and inconsistently resolved. Rennie et al., (1989) indicated that whereas all variants of ADH could be separated on the polyacrylamide/starch system used by Bult et al., (1989), results were inconsistent with the starch gel system.

Haack et al., (1992) reported that sampling imbibed cotyledons at 6 h after planting on germination paper resulted in greater intensity of band 1 which was less clearly resolved when sampling was done at 12 h, 18 h, or 24 h after planting. They also reported the unreliable resolution of band 1 when sampling was done at 36 h, 42 h, or 48 h after planting, and mentioned the existence of unexplained non-genetic variability between runs which needed further investigation. It was speculated by Haack et al., (1992), that soybean ADH may be a metalloenzyme, and a strong chelator such as citric acid (a component of the electrode buffer) could interact with a divalent ion associated with the enzyme, thereby altering electrophoretic mobility. Use of a de-chelator such as ethylene diaminetetraacetic acid (EDTA) might increase consistency of separation and resolution. Wendel and Weeden (1989) pointed out that a great amount of variability can exist among different lots of starch regarding cooking and electrophoretic qualities. This can be an important source of inconsistencies in results. The consistent resolution of band 1 would be particularly useful in scoring for Adh_1 (one of the loci of Linkage Group 8) in genetic linkage studies.

The objective of this study was to develop a procedure of starch gel electrophoresis that would consistently separate and resolve all seven bands of

soybean ADH.

Materials and Methods

Sampling procedure: To develop a procedure of identifying adh_1adh_1 lines for further genetic linkage studies, the procedure of Haack et al., (1992) was followed with modifications. Using experimental lines as well as the eight cultivars used in their study (Beeson, BSR 101, Cayuga, Cutler, Jefferson, Lincoln, Swift, and Wye - seed obtained from R. L. Nelson), imbibed cotyledons (incubated in darkness at 30 °C) were sampled at 6 h, and between 16 h - 24 h after planting. Cotyledon samples were ground in 30 µl of regular homogenization buffer [16.7 g of sucrose plus 8.3 g sodium ascorbate in 100 ml of deionized distilled water (ddH_2O)], pH 6.5, and regular buffer with 0.003 g ml^{-1} EDTA added, in micro-centrifuge tubes on ice/ice water. Samples were centrifuged for 90 sec at 12,000 rpm in a Fisher Micro-Centrifuge model 235B. Pre-punched electrophoretic wicks (11 x 2.0 mm Whatman # 2 filter paper) were placed into each sample to absorb the supernatant before freezing/re-freezing for later use.

Buffer and gel preparation: Decreasing the amount of citric acid from 1.5 to 0.55 g L^{-1} ddH_2O increased pH of electrode buffer from 6.5 to 6.8. In this study electrode buffers were prepared using both 0.55 g citric acid L^{-1} ddH_2O, and 1.5 g citric acid L^{-1} ddH_2O, with 10.1 g L^{-1} L-histidine.

Samples of different starch lots from SIGMA Chemical Co. were tested and the best (clear band resolution and separation without smearing) lot use for this study. To develop a procedure which would give consistent and reliable resolution and separation, gels were prepared with 500 ml of three different gel buffers (1 part electrode buffer to 1 part, 3, and 6 parts ddH_2O), and 60.7 g (12.14% w/v) of hydrolysed potato starch. Haack et al., (1992) used a 1 part electrode buffer to 3 parts ddH_2O. To characterize different banding patterns from soybean cultivars and experimental lines, a gel buffer mix of 1 part electrode buffer (1.5 g citric acid L^{-1} ddH_2O, with 10.1 g L^{-1} L-histidine) to 1 part of ddH_2O gave superior results and was used. For each gel, a starch suspension was made in 150 ml of gel buffer, while the remaining 350 ml was cooked in a KENMORE microwave on high for 230 sec before adding it to the starch suspension and cooking on high for approximately 95 sec. Gels were cooled to room temperature before being wrapped in Saran Wrap, and left at room temperature overnight. Gels were cooled in a refrigerator at 5 °C for 15 to 20 min before loading with wicks.

Electrophoresis and staining: Gels were run for 4.0 h, 5.5 h, 7.5 h, and 11.0 h at 12 W, 9.5 W, 7.5 W, and 4.5 W constant wattage per gel, respectively. One gel, two gels, three gels, and four gels at a time were connected to a separate power source (Fisher Biotech Electrophoresis System, model FB701). Haack et al., (1992) used 9.5 W for 5.5 h. After electrophoresis, double-thickness bottom slices were stained in 55 ml/gel of stain solution [50 ml buffer # 2 of pH 8.0 which comprises 24.23 g Tris L^{-1} ddH_2O, approximately 42.0 ml concentrated HCl, 5 drops of 95% ethanol, 2 ml (25 mg/50 ml) nicotinamide adenine dinucleotide (NAD), 2 ml (25 mg/50 ml) nitro blue tetrazolium (NBT), and 1.0 ml (20 mg/50 ml) phenazine methosulfate (PMS)] overnight in darkness in an incubator at 38 °C. We did not include agar dissolved in Tris-HCL in the stain solution as Haack et al., (1992) used.

Gels were scored after 24 h, because, while bands 2 through 7 appeared within 30 min to 7 h, band 1 appeared much later.

Mention of trademark or proprietary product by the USDA or Iowa State University does not imply its approval to the exclusion of other products that may also be suitable.

Results and Discussion

Preliminary studies in our laboratory confirmed the observation by Wendel and Weeden (1989) regarding the alarming amount of variation from one starch lot to another with respect to cooking and electrophoretic quality. We frequently observed smearing between bands and inconsistent resolution of band 1 when gels were prepared with a particular lot of starch from SIGMA. Band migration and separation was poor when four gels were run connected to the same power source.

Table 1 illustrates results of band resolution. Our study revealed that although a double band 1 appeared for some experimental samples ground in homogenization buffer with EDTA added, the addition of EDTA to regular homogenization buffer did not seem to make any difference in migration pattern of bands, and resolution of band 1 in particular. Although bands were closer together, the best and most consistent resolution of band 1 was observed when gels were prepared with a 1 part electrode buffer (1.5 g citric acid L^{-1}) to 1 part ddH_2O gel buffer and electrophoresed for 7.5 h at 7.5 W regardless of whether EDTA was added to the homogenization buffer. This suggests that some other factor, rather than chelation as speculated by Haack et al., (1992), may be responsible for inconsistent electrophoretic mobility. Use of 0.55 g citric acid L^{-1} to prepare electrode buffer resulted in greatest band separation.

Contrary to the findings of Haack et al., (1992), we observed smearing between bands, and band 1 was inconsistently observed on gels prepared with the 1:3 gel buffer with both 0.55 g and 1.5 g citric acid L^{-1} ddH_2O.

Table 1. Resolution of ADH using different homogenization buffers, electrode buffer compositions, gel buffer compositions, and run times.

				Appearance of band 1 Homogenization buffer	
Electrode buffer	Gel buffer†	Time (hours)	Wattage (W)	Without EDTA	With EDTA
0.55g CA/L‡	1:1	4.0	12.0	-	-
		4.5	11.0	-	-
		5.5	9.5	-	-
		7.5	7.5	-	-
	1:3	4.0	12.0	-	-
		4.5	11.0	-	-
		5.5	9.5	+§	+§
		7.5	7.5	-	-
	1:6	4.0	12.0	-	-
		4.5	11.0	-	-
		5.5	9.5	-	-
		7.5	7.5	-	-
1.50g CA/L	1:1	4.0	12.0	-	-
		4.5	11.0	-	-
		5.5	9.5	-	-
		7.5	7.5	+	+
	1:3	4.0	12.0	-	-
		4.5	11.0	-	-
		5.5	9.5	+§	+§
		7.5	7.5	-	-
	1:6	4.0	12.0	-	-
		4.5	11.0	-	-
		5.5	9.5	-	-
		7.5	7.5	-	-

† 1 part electrode buffer to 1-, 3-, and 6- parts ddH_2O; ‡ CA = citric acid; § band 1 inconsistently resolved; - no band 1.

We observed five banding patterns. A 5- band pattern (type 2), similar to that described by Gorman and Kiang (1977) and Haack et al., (1992), was observed in addition to 4-, 6-, and 7- band patterns (Figure 1). The 4- and 7- band patterns observed in this study were different from those reported by Gorman et al., (1982), and Haack et al., (1992). Their 4- band pattern (type 3) did not include bands 1, 4 (crescent-shaped), and 5; ours did not include bands 1, 2 and 4. The difference between their 7- band pattern and ours was the absence of band 4 in ours and the absence of double band 1 or 2 in theirs (Figure 1). The 5- band pattern, and 7- band pattern (with a double band 1, or 2) were mostly observed in the experimental lines used. The double band 2 pattern showed up a number of times. A double band 1 was observed only once in one seed of an experimental line. The inheritance pattern of this double band could not be studied because that seedling was lost. Degradation products can sometimes be mistaken for bands, resulting in the scoring of double-bands. However, the existence of double-bands and even multiple-bands is possible. These have been reported for many plant isozymes including shikimate dehydrogenase (Harry, 1986; Jarret and Litz, 1986; Tanksley, 1984; Weeden, 1984).

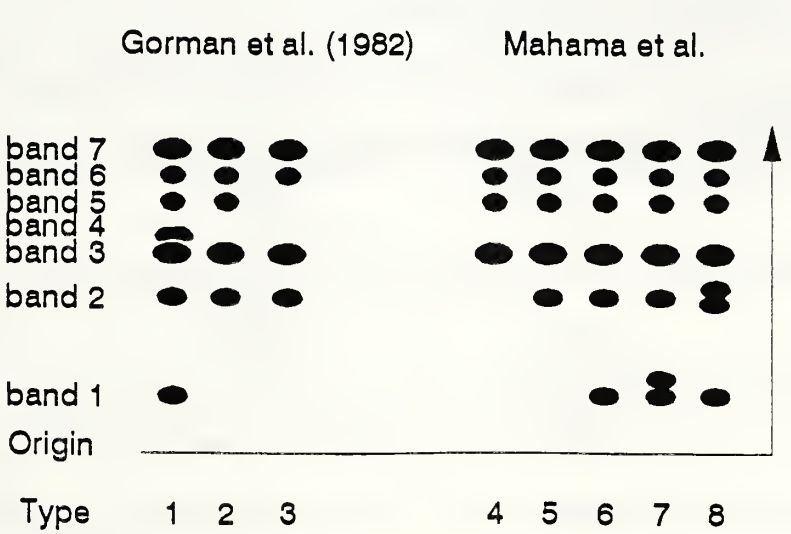

Figure 1. Observed banding patterns of ADH.

Seven bands were observed for Beeson, Cutler, and Swift; four bands were observed for Cayuga, Jefferson, Lincoln, and Wye, and a six-band pattern was observed for BSR 101 (Table 2). The banding patterns observed for Beeson, Cayuga, Cutler, and Jefferson were slightly different from those reported by Gorman et al.,

(1982), and Haack et al., (1992). Although band 1 was present in Beeson, Cutler, and Swift, band 4 (crescent-shaped), of the 7- band pattern (type 1) (Figure 1) was not observed in our study. Also 4 bands were observed for Wye, however, the pattern was different from that reported by Gorman et al., (1982) and Haack et al., (1992). While Gorman et al., (1982), and Haack et al., (1992) observed the same banding pattern for Lincoln, we observed a different pattern. Similarly, while Haack et al., (1992) observed 7 bands for BSR 101, we observed 6 bands (band 1 inclusive) for the same cultivar. These observations suggest the possible existence of mobility variants. A mobility variant (Adh3) was reported by Yu and Kiang (1993). The non-genetic variability between runs, reported by Haack et al., (1992), may be due to factors which include gel preparation methods, starch used, and power source. In spite of the differences in banding patterns observed in this study compared with the reports of Gorman et al., (1982) and Haack et al., (1992), resolution of band 1 is of greatest importance. Band 1 was observed in the two earlier studies cited as well as in this study. The difference, however, is that while band 1 was inconsistently resolved in those studies, it was consistently resolved in our study. Since resolution of band 1 was consistent with the use of 1 part electrode buffer (1.5 g citric acid L^{-1}) to 1 part ddH_2O to prepare gels, and running at 7.5 W for 7.5 h, this procedure was used for the identification of adh_1adh_1 lines in other studies.

Table 2. Number of ADH bands resolved for eight different cultivars, and experimental lines.

	Haack et al., (1992)	Gorman et al., (1989)	Mahama et al.,
No. of bands		Cultivar	
7	Beeson BSR 101 Cutler Swift	Beeson Cutler	Beeson Cutler Swift
6	--	--	BSR 101
5	Lincoln Wye	Lincoln Swift	Experimental lines
4	Cayuga Jefferson	Cayuga Jefferson Wye	Cayuga Lincoln Jefferson Wye

Conclusions

Using the right kind of starch is critical for consistent resolution of soybean ADH, particularly band 1. The main modifications which yielded the consistency reported herein include the following: use of good starch, preparation of gels with a gel buffer mix of 1 part electrode buffer (1.5 g citric acid L^{-1}) to 1 part ddH_2O, and running gels at 7.5 W for 7.5 h. The observations of this study compared with those of Gorman et al., (1982) and Haack et al., (1992) suggest the possible existence of more banding patterns than those reported so far. Although degradation products could be mistaken for bands, the regular appearance of a double-band 2 in our study seem to suggest its real existence. These need further investigation.

References.

Bult, C.J., Y.T. Kiang, Y.C. Chiang, H.J.Y. Doong, and M.B. Gorman. 1989. Electrophoresis methods for soybean genetic studies. Soybean Genet. Newsl. 16:175-187.

Cardy, B. J. and W.D. Beversdorf. 1984. A procedure for the Starch Gel Electrophoretic Detection of Isozymes of Soybean [Glycine max (L.) Merr.]. Dep. Crop Sci. Tech. Bull. 119/8401, Univ. of Guelph, Guelph, Ontario. p 1-29.

Gorman, M.B., Y.T. Kiang, and R.G. Palmer. 1982. Electrophoretic classification of the early maturity groups of named soybean cultivars. Soybean Genet. Newsl. 10:143-156.

Gorman, M.B. and Y.T. Kiang. 1977. Variety specific electrophoretic variants of four soybean enzymes. Crop Sci. 17:963-965.

Haack, K.S., T.D. Couch, J. Imsande, and R.G. Palmer. 1992. A starch gel electrophoretic procedure for resolving soybean alcohol dehydrogenase band 1. Soybean Genet. Newsl. 19:92-97.

Harry, D.E. 1986. Inheritance and linkage of isozyme variants in incense-cedar. J. Hered. 77:261-266.

Jarret, R.L. and R.E. Litz. 1986. Enzyme polymorphism in Musa acuminata Colla. J. Hered. 77:183-188.

Rennie, B.D., M.L. Thorpe, and W.D. Beversdorf. 1989. A Procedural Manual for the Detection and Identification of Soybean [Glycine max (L.) Merr.] Isozymes Using the Starch Gel Electrophoretic System. Dep. Crop Sci. Tech. Bull. Univ. of Guelph, Guelph, Ontario, Canada. p 1-54.

Tanksley, S.D. 1984. Linkage relationships and chromosomal locations of enzyme-coding genes in pepper, Capsicum annuum. Chromosoma 89:352-360.

Weeden, N.F. 1984. Distinguishing among white seeded bean cultivars by means of allozyme genotypes. Euphytica 33:199-208.

Wendel, J.L. and N.F. Weeden. 1989. Visualization and Interpretation of Plant Isozymes. p.5-45. In D. E. Soltis and P. S. Soltis (eds.), Isozymes in plant biology. Dioscorides Press, Portland.

Yu, H. and Y.T. Kiang. 1993. Inheritance and Genetic Linkage Studies of Isozymes in Soybean. J. Hered. 84:489-492.

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Mapping of the microsomal and plastid omega-3 fatty acid desaturases in soybean [Glycine max (L.) Merr.]

Soybean produces seed oil in which the fatty acid composition affects the nutritional value as well as its physical and chemical characteristics (Neff et al., 1992). The polyunsaturated fatty acid a-linolenate(18:3$^{\omega 3,6,9}$) is synthesized by plants, but not by most other higher eukaryotes. This fatty acid is an essential component of human nutrition because in mammals it acts as a precursor to membrane lipids and families of signaling molecules (Smith and Borgeat, 1985). It is also commercially important because it has been identified as the unstable component of soybean oil responsible for undesirable odors and flavors (Liu and White, 1992). For these reasons, variation in the linolenic acid content has been a target for genetic selection. The application of conventional breeding methods, coupled in some cases with mutagenesis, has resulted in the production of new lines with desirable alterations in the fatty acid composition of the seed oil (Hammond and Fehr, 1983). Most of the genetic variation in seed lipid fatty acid composition appears to involve the presence of an allele of a gene that disrupts normal fatty acid metabolism and leads to an accumulation of intermediate fatty acid products in seed storage lipids. It is unclear, however, in these mutants what structural genes have been mutated in existing public soybean germplasm.

The biosynthesis of linolenic acid is the result of a desaturation reaction catalyzed by a membrane associated omega-3 desaturase which introduces the third double bond into the 18-carbon fatty acid. In leaf tissue there are two distinct pathways for polyunsaturated fatty acid biosynthesis, one located in the microsomes and the other located in the plastid membranes. In Arabidopsis thaliana, the plastid ω-3 fatty acid desaturations are controlled by the FAD 7 locus (Browse and Somerville, 1991). The major enzyme responsible for the synthesis of seed linolenic acid is the microsomal ω-3 linoleate desaturase. Its activity is controlled in Arabidopsis by the FAD 3 locus (Lemieux et al., 1990) which should be equivalent to one of the FAN loci in soybean. The objective of this study was to genetically map the microsomal and plastid ω-3 linoleate desaturases in the USDA-ARS G. max/G. soja population utilized

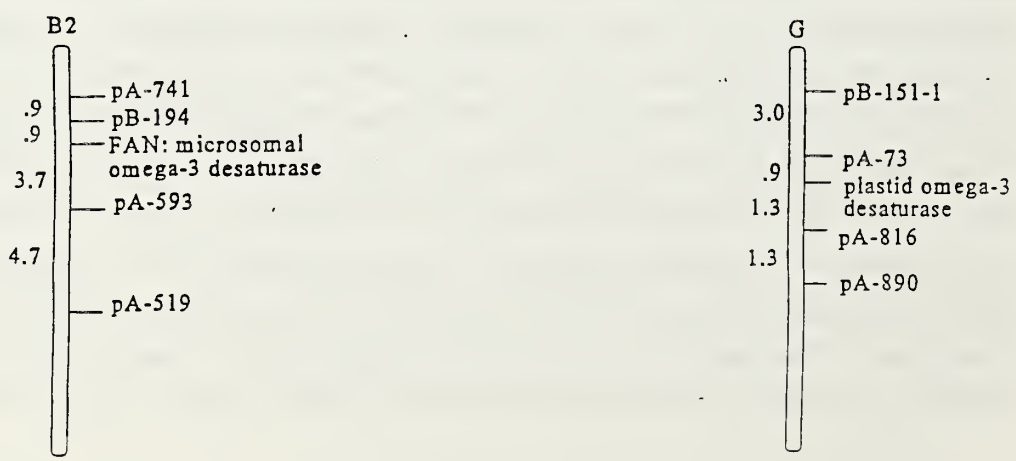
in constructing the public soybean map.

Materials and Methods:

DNA Gel-Blot Analysis: Total genomic DNA from young soybean leaves was isolated as previously described by (Anderson et al., 1992). Restriction digests, electrophoresis on agarose gels, Southern blots, hybridizations, and autoradiography was according to (Berantzky and Tanksley, 1986) with minor modifications. Ten micrograms of parental DNA was digested with EcoRI, Hind III, Taq I, Eco RV and Dra I. DNA from the parental genotypes was analyzed by gel-blot hybridization to the cDNA's coding for the microsomal and plastid ω-3 linoleate desaturase (Yadav et al., 1993) to identify polymorphisms. When polymorphisms were identified, DNA from each segregant in the USDA-ARS G. max/G. soja F_2 derived population was restricted with Eco RI and Dra I and analyzed by gel-blot hybridization with both cDNA's. The segregation for the two cDNA's was incorporated with the existing data set of RFLP markers and mapped using the program Mapmaker (Lander et al., 1987).

Results

A microsomal ω-3 linoleate desaturase mapped to linkage group B2. This is consistent with the placement of fan (A5) at the same location in another population (Brummer et al., 1995). This suggests that the low linolenic acid phenotype obtained with the fan (A5) locus is the result of a mutation in the microsomal ω-3 linoleate desaturase gene. The plastid ω-3 linoleate desaturase maps to linkage group G.



Discussion

Soybean produces seed oil in which the fatty acid composition is not ideally suited for the intended use. The application of conventional breeding methods, coupled in some cases with mutagenesis, has resulted in the production of new lines with desirable alterations in the fatty acid composition of the seed oil. Biochemical and genetic studies of these mutants are important to our understanding of the 18:2 desaturation step because the enzyme is an integral membrane protein that has been difficult to solubilize and, therefore, to investigate by traditional enzymological methods. In the absence of a purified enzyme, genetic techniques can be an alternative means to study the relevant genetic locus. However, it seems likely that, because of the inherent limitations of mutation breeding, many other desirable changes in seed oil fatty acid composition may require the directed application of genetic engineering methods. Presently, specific information about the biochemistry and regulation of lipid metabolism have made it possible to predict the result of the introduction of one or a few genes that might usefully alter seed lipid synthesis. This would allow scientists to design new products that can provide expanded or new markets for excess agricultural output.

References

Anderson, J.A., Y. Ogihara, M.E. Sorrells, and S.D. Tanksley. 1992. Development of a chromosomal arm map for wheat based on RFLP markers. Theor. Appl. Genet. 83:1035-1043.

Bernatzky, R., and S.D. Tanksley. 1986. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898.

Brummer, E.C., A.D. Nicolle, J.R. Wilcox, and R.C. Shoemaker. 1995. Mapping the fan(A5) locus. Controlling fatty acid content in sobean oil. J. Heredity (In Press).

Browse, J., and C. Somerville. 1991. Glycerolipid Synthesis: Biochemistry and Regulation. Ann Rev Plant Physiol Plant Mol Biol. 42:467-506.

Hammond, E.G., and W.R. Fehr. 1983. Registration of A5 germplasm line of sobean. Crop Sci. 23:192.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. Mapmaker: and interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174-181.

Lemieuz, B.M., C. Somerville, and J. Browse. 1990. Mutants of Arabidopsis with alterations in seed lipid fatty acid composition. Theor. App. Gen. 80:234-240.

Liu, H.R., and P.J. White. 1992. Oxidative Stability of Soybean Oils with Altered Fatty Acid Composition. JAOCS 69:528-532.

Neff, W.E., E. Selke, T.L. Mounts, W., Rinsch, E.N. Frankel, and M.A.M. Zietoun. 1992. Effect of Triacylglycerol Composition and Structures on Oxidative Stability of Oils from Selected Soybean Germplasm. JAOCS 69:111-118.

Smith, W.L., and P. Borgeat P. 1985. The eicosanoids: Prostaglandins, thromboxanes, leukotrienes and hydroxy-eicosaenoic acids. In Biochemistry of Lipids and Membranes, DE Vance and JE Vance, eds (Menlo Park, CA: Benjamin/Cummings) pp. 325-360.

Yadav, N., A. Wierzbicki, M. Aegerter, C.S. Caster, P.G. Luis, A.J. Kinney, W.D. Hitz, R. Booth, B. Schweiger, K.L. Stecca, S.M. Allen, M. Blackwell, R.S. Reiter, T.J. Carlson, S.H. Russell, K.A. Feldmann, J. Pierce, and J. Browse. 1994. Cloning of Higher Plant Omega-3 Fatty Acid Desaturases. Plant Physiol 1993(103):467-476.

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Genetic analysis of nonnodulating soybean mutants in a hypernodulated background

Nodulation has been successfully manipulated in soybean (Glycine max) by mutagenesis, leading to supernodulated or hypernodulated phenotypes (Akao and Kouchi, 1992; Buzzell et al., 1990; Carroll et al., 1985; Gremaud and Harper, 1989) and to nonnodulated phenotypes (Carroll et al., 1986; Harper, 1989). Most of the supernodulated or hypernodulated mutants which have been genetically characterized are controlled by single recessive genes (Buzzell et al., 1990; Delves et al., 1988; Kokubun and Akao, 1994; Pracht et al., 1992). Exceptions are the nts 1116 hypernodulated mutant from Bragg which was described as being "leaky" (Delves et al., 1988) and the E714 supernodulated mutant from Elgin 87 for which preliminary results indicated possible involvement of two recessive genes (Buzzell et al., 1990).

The nonnodulating mutants from Gresshoff's and Harper's laboratories are controlled by either one or two recessive genes (Mathews et al., 1989; Pracht et al., 1993). Two recessive nonnodulating genes (rj_5, rj_6) were identified as being necessary for the nonnodulating phenotype of the NN5 mutant selected from mutagenized Williams soybean, and both genes were also confirmed to be present in nod 139 (Pracht et al., 1993), a nonnodulating mutant selected from mutagenized Bragg (Carroll et al., 1986). A previously identified rj_1 gene was identified in a spontaneous nonnodulating mutant (Williams and Lynch, 1954). Mathews et al. (1990) reported that nonnodulation epistatically suppresses the supernodulation genotype. The terms supernodulation and hypernodulation were proposed by Gresshoff and Delves (1986) to describe extensive and intermediate degrees of nodulation, respectively, compared to the normally nodulated parent. Based on direct comparison of these lines, we do not currently believe that a clear distinction exists between supernodulating and hypernodulating lines, and hence in subsequent reference we use the term hypernodulation for all increased nodulation mutants.

We recently isolated two plants from a hypernodulated soybean mutant (NOD3-7) which were nonnodulated (designated 3-7 17b and 3-7 21-2, collectively referred to as 3-7). These were rescued, grown to maturity, and evaluated through several generations. These lines bred true and were used in the present study to genetically

characterize expression of nonnodulation in a hypernodulated background.

Materials and Methods:

Crosses were made involving two of the selected nonnodulating lines arising from the hypernodulated NOD3-7 mutant, and two previously characterized nonnodulating mutants (NN5 and L65-1274). The hypernodulating trait of the NOD3-7 parent line is known to be controlled by a single recessive gene (Harper, unpublished) which is allelic to that in NOD 4 (Pracht et al., 1992). We are recommending that the recessive hypernodulation gene which was temporarily designated as rjh by Pracht et al. (1992) be designated as rj_7. This request has been approved by the Soybean Genetics Committee and rj_7 is used in this paper to designate the recessive hypernodulation gene. The NN5 line carries the rj_5 and rj_6 recessive genes (Pracht et al., 1993), while the Harosoy nonnodulating line (L65-1274) carries the rj_1 recessive gene (Williams and Lynch, 1954). The putative genotypes of the four parents evaluated are: NN5, $Rj_1Rj_1rj_5rj_5rj_6rj_6Rj_7Rj_7$; L65-1274, $rj_1rj_1Rj_5Rj_5Rj_6Rj_6Rj_7Rj_7$; and the two 3-7.. lines, $Rj_1Rj_1rj_5rj_5rj_6rj_6rj_7rj_7$. The F_1 seeds were inoculated with a commercial, peat based source of Bradyrhizobium japonicum (Urbana Laboratories, St. Joseph Missouri) and planted in quartz gravel beds in the greenhouse (Harper, 1971). Seeds were germinated with tap water without additional nutrients. At two weeks after inoculation and planting in the greenhouse, seedlings were removed from the quartz gravel beds and visually evaluated for nodulation. After classifying, seedlings were transplanted back into the gravel beds and a urea-based nutrient (Pracht et al., 1993) solution was provided until maturity. The F_2 seeds were harvested from individual F_1 plants, inoculated, and planted in the same greenhouse gravel beds. Again after two weeks, F_2 seedlings were evaluated and transplanted to the field for advance to the F_3. Individual F_2 plants, tracing back to specific nodulation phenotypes, were harvested and $F_{2:3}$ families were again visually evaluated in the greenhouse at the seedling stage for nodulation expression.

Results and Discussion

The F_1 plants from crosses between NN5 and the two nonnodulated lines (3-7 17b and 3-7 21-2, collectively designated 3-7) were all nonnodulated (data not shown). Nonnodulation was confirmed in the F_2 and indicated that the nonnodulating 3-7. lines carried both recessive genes (rj_5, rj_6) previously identified in NN5 (Pracht et

al., 1993). The above results, however, would also be obtained if the anticipated crosses were selfs. This does not seem likely since 10 crosses were made and 19 F_1 plants were tested. However, we were unable to verify true hybrids since no other definitive markers are available; both lines were derived from the Williams background.

Crosses of the Harosoy nonnodulating isoline (rj1) with the 3-7.. lines were all nodulated in the F1 (Table 1). This is consistent with previous results (Pracht et al., 1993) indicating that the Harosoy nonnodulating line was controlled by a different recessive gene than found in NN5. Evaluation of F2 progeny from crosses of the Harosoy nonnodulating line with the 3-7.. lines resulted in segregation into three classes, normally nodulated, hypernodulated, and nonnodulated in a 135 : 45 : 76 ratio. The putative basis for this ratio is that rj1rj1 or the combination of rj5rj5rj6rj6 both result in nonnodulation and epistatically suppress rj7rj7. Hypernodulation occurs when rj7 is homozygous recessive and neither rj1 nor the combination of rj5 + rj6 are homozygous recessive. Remaining combinations of the 256 possible will be normally nodulated. Both crosses (with four and three F1 plants) evaluated resulted in significant probability of fitting the model, and the pooled chi square probability was quite high. The results confirmed that neither of the nonnodulation genes in the 3-7.. lines was allelic to rj1 present in the Harosoy nonnodulated lines. This also provides additional confirmation of the genetic model described by Pracht et al., (1993) in crosses between NN5 and the Harosoy nonnodulating isoline.

The F_2 plants were further evaluated as $F_{2:3}$ families for nodulation classes (Table 2). Evaluation of nonnodulating F_2's resulted in nonnodulating $F_{2:3}$ families, verifying that these F_2 plants were homozygous for nonnodulation. As expected, the hypernodulated plants in F_2 were classified into two classes in the $F_{2:3}$ families (either homozygous hypernodulated or segregating for nonnodulated + hypernodulated). The normally nodulated F_2 plants resulted in four classes in the $F_{2:3}$ families: (1) homozygous normal; (2) segregation for normal nodulation and hypernodulation; (3) segregation for normal nodulation and nonnodulation; and (4) segregation for normal nodulation, nonnodulation, and hypernodulation. The low probability level (0.08) for fitting the proposed four gene model is likely due to misclassification. It is known that vigorous seedling growth is needed to provide full expression of nodulation and several conditions could curtail growth, such as seed coat impermeability which delays imbibition and germination, seed borne pathogens which can affect root growth, etc. Because it was felt that the visual classification may lead to incorrect placement of some normally nodulated seedings into the nonnodulating class, and

some hypernodulating lines into the normally nodulated class, data were combined over specific classes and retested against expected ratios. This resulted in higher probabilities that the model was correct (Table 2), and we conclude that the genotypes of the nonnodulated 3-7 lines are both $\underline{Rj_1}\underline{Rj_1}\underline{rj_5}\underline{rj_5}\underline{rj_6}\underline{rj_6}\underline{rj_7}\underline{rj_7}$. The $\underline{rj_1}$ nonnodulation allele and the combination of $\underline{rj_5}$ and $\underline{rj_6}$ nonnodulation alleles epistatically suppress the $\underline{rj_7}$ hypernodulation allele. Both of the 3-7 nonnodulating lines likely arose from a single spontaneous mutational event in an earlier generation of the NOD3-7 hypernodulated line and are likely identical. Seeds of these mutants are available.

Table 1. Segregation among F_2 plants from crosses of two nonnodulating mutants of Williams with nonnodulating L65-1274 (a near isoline of Harosoy that is homozygous for $\underline{rj_1}$) (Bernard, 1974).

Cross	F_1 plant No.	F_1	F_2 Normal	F_2 Hyper	F_2 Nonnod	Calculated χ^2 (135:45:76)	P
HarNN X 3-7 17b	1	All Nod	15	9	7	2.95	0.23
	2	All Nod	20	7	8	0.80	0.67
	3	All Nod	24	10	16	0.47	0.79
	4	All Nod	35	8	14	1.72	0.42
HarNN X 3-7 21-2	1	All Nod	34	9	24	1.55	0.45
	2	All Nod	41	17	21	0.97	0.62
	3	All Nod	80	16	46	3.92	0.14
	Total		249	76	136	0.46	0.79

Table 2. Phenotypes of F_3 families derived from normally nodulated (Nor), hypernodulated (Hyp), and nonnodulated (NN) F_2 plants from crosses between nonnodulating mutants of Williams (3-7 17b, 3-7 21-2) and nonnodulating Harosoy (L65-1274). Seeds were inoculated with Bradyrhizobium japonicum and grown 2 weeks before visual evaluation of nodulation reaction.

F_2	F_3 family	Exp.	Obs.	χ^2 Prob	F_3 fam.	Exp.	Obs.	χ^2 Prob	F_3 Fam.	Exp.	Obs.	χ^2 Prob
Nor	Homozygous Nor	14	18	0.08	Homo. Nor	14	18	0.28	Homo. Nor	14	18	0.56
	Seg. Nor/Hyp	28	28		Combined[1]	257	253		Seg. Nor/hyp	28	28	
	Seg. Nor/NN	76	92						Combined[2]	225	229	
	Seg. Nor/NN/Hyp	152	133									
Hyp	Homozygous Hyp	5	6	0.98								
	Seg. NN/Hyp	27	33									
NN	Homozygous NN	all	all									

[1]Combined over three classes; Seg. Nor/Hyp + Seg. Nor/NN + Seg Nor/NN/Hyp.
[2]Combined over two classes; Seg. Nor/NN + Seg. Nor/NN/Hyp.

References

Akao, S., and H. Kouchi. 1992. A supernodulation mutant isolated from soybean cultivar Enrei. Soil Sci. Plant Nutr. 38:183-187.

Buzzell, R.I., B.R. Buttery, and G. Ablett. 1990. Supernodulation mutants in Elgin 87 soybean. pp. 726. In Gresshoff, P.M., B. G. Rolfe, G. Stacey, and W.E. Newton (eds.). Nitrogen Fixation: Achievements and Objectives. Chapman and Hall, New York.

Carroll, B.J., D.L. McNeil, and P.M. Gresshoff. 1985. A supernodulation and nitrate-tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78:34-40.

Carroll, B.J., D.L. McNeil, and P.M. Gresshoff. 1986. Mutagenesis of soybean [Glycine max (L.) Merr.] and the isolation of non-nodulating mutants. Plant Sci. 47:109-114.

Delves, A.C., B.J. Carroll, and P.M. Gresshoff. 1988. Genetic analysis and complementation studies on a number of mutant supernodulating soybeans. J. Genetic. 67:1-8.

Gremaud, M.F. and J.E. Harper. 1989. Selection and initial characterization of partially nitrate tolerant nodulation mutants of soybean. Plant Physiol. 89:169-173.

Gresshoff, P.M. and A.C. Delves. 1986. Plant genetic approaches to symbiotic nodulation and nitrogen fixation in legumes. pp 159-206. In A.O. Blonstein & P.J. King (ed.). A Genetic Approach to Plant Biochemistry. Plant Gene Research. Springer-Verlag, New York.

Harper, J.E. 1971. Seasonal nutrient uptake and accumulation patterns in soybeans. Crop Sci. 11:347-350.

Harper, J.E. 1989. Nitrogen metabolism mutants of soybean. pp. 212-216. In. A.J. Pascale (ed.). Proceedings of World Soybean Research Conference IV. Buenos Aires, Argentina.

Kokubun, M. and S. Akao. 1994. Inheritance of supernodulation in soybean mutant En6500. Soil Sci. Plant Nutr. 40:715-718.

Mathews, A., B.J. Carroll, and P.M. Gresshoff. 1989. A new nonnodulation gene in soybean. J. of Heredity. 80:357-360.

Mathews, A., B.J. Carroll, and P.M. Gresshoff. 1990. The genetic interaction between non-nodulation and supernodulation in soybean: an example of developmental epistasis. Thero. Appl. Genet. 79:125-130.

Pracht, J.E., C.D. Nickell, and J.E. Harper. 1992. Genetic analysis of a hypernodulating mutant of soybean. Soybean Genetics Newsl. 20:107-111.

Pracht, J.E., C.D. Nickell, and J.E. Harper. 1993. Genes controlling nodulation in soybean: Rj_5 and Rj_6. Crop Sci. 33:711-713.

Williams, L.F., and D.L. Lynch. 1954. Inheritance of a non-nodulating character in the soybean. Agron. J. 46:28-29.

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PI 317334-B not non-sensitive to photoperiod

Massey, Bernard, and Hittle reported in 1981 that P.I. 317334B was non-sensitive to photoperiod. Field plantings were made at Urbana, IL and at Isabela, Puerto Rico. In additdion to the plantings under natural photoperiod, a second planting hd photperiod extended to 24 hours using incandescent bulbs. P.I. 317334B showed almost no difference in days to flowering or to maturity under either lighting system.

In view of my skepticism that we actually have genotypes within Glycine max (L.) Merr. that are non-sensitive to photoperiod, I planted seed of Williams and P.I. 317334-B at Stoneville, MS on May 13, 1994. If P.I. 317334-B were non-sensitive to photoperiod, it would have required a greater number of days to early bloom at Stoneville than would Williams. Both strains began flowering on 14 June and were considered mature 29 August. Based upon these results, P.I. 317334-B is equal in sensitivity to photoperiod as is Williams.

Differences reported earlier among germplasm lines of Group III maturity in days to flowering or maturity may have been from responses to light qualtiy or light intensity.

References

Massey, C.R., R.L. Bernard, and C.N. Hittle. 1981. Variation in photoperiod sensitivity for time of flowering and maturity among soybean strains of Maturity Group III. Crop Sci. 21:833-836.

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Analysis of the apparent w_4 locus in soybean line P.I. 81.763

Flower color of soybean [Glycine max (L.) Merr.] is influenced by the epistatic gene interaction of several loci, including W_1, W_3, W_4, W_m and W_p. Dominant alleles at the W_1 and W_4 loci indicate the presence of anthocyanin biosynthesis, producing purple pigmentation in flowers and hypocotyls (Groose et al., 1988; Hartwig and Hinson, 1962). White flowers arise due to absence of anthocyanin caused by genotypes homozygous recessive at the w_1 locus. The intensity of pigmentation is modified by the W_3 locus (Groose and Palmer, 1991) so that the genotype $W_1\_$, $W_3\_$, $W_4\_$ produces dilute-purple and the genotype $W_1\_$, w_3w_3, w_4w_4 conditions near-white flower phenotypes (Groose et al., 1988). In addition, magenta flowers arise from the genotype $W_1\_$, w_3w_3, $W_4\_$, w_mw_m (Buzzell et al., 1977) and pink flowers are conditioned from the $W_1\_$, w_3w_3, $W_4\_$, w_pw_p genotype (Stephens and Nickell, 1992).

Recently, an apparent novel source of near-white flower color was identified in P.I. 81.763. Analysis of the inheritance of this novel source will determine if linkages exist between the genes that control this near-white phenotype and other known loci controlling flower color.

Materials and Methods

The source of near-white phenotype from P.I. 81.763, was obtained from R.L. Bernard, USDA-ARS Urbana, IL. This was backcrossed into the cultivars Clark and Harosoy to create Clark w_x (L70-4448) and Harosoy w_x (L68-705), both selected for homozygosity at the w_x locus. Isolines of Harosoy, with different flower color alleles (Bernard, 1974), were utilized as females. The Clark w_x was used as a pollen source and crossed with each Harosoy isoline (Table 1). This allowed for easy identification of crosses as the Harosoy isolines have gray pubescence and the Clark w_x has tawny pubescence. Therefore, F_1 plants with tawny pubescence would indicate a successful cross. Flower color and pubescence type of F_2 plants were examined and documented in Table 2.

Table 1. Genotype and phenotypes of parental isolines

Designation	Genotype	Pubescence1	Flower phenotype
L70-4448 (Clark $\underline{w_4}$)2	$\underline{W_1W_1}$, $\underline{w_3w_3}$, $\underline{w_4w_4}$, $\underline{W_mW_m}$	T	near-white
L68-705 (Harosoy $\underline{w_4}$)2	$\underline{W_1W_1}$, $\underline{w_3w_3}$, $\underline{w_4w_4}$, $\underline{W_mW_m}$	G	near-white
Harosoy 63	$\underline{W_1W_1}$, $\underline{w_3w_3}$, $\underline{W_4W_4}$, $\underline{W_mW_m}$	G	purple
L72-1078	$\underline{W_1W_1}$, $\underline{W_3W_3}$, $\underline{w_4w_4}$, $\underline{W_mW_m}$	G	dilute-purple
L72-1138	$\underline{W_1W_1}$, $\underline{w_3w_3}$, $\underline{w_4w_4}$, $\underline{W_mW_m}$	G	near-white
T235	$\underline{W_1W_1}$, $\underline{w_3w_3}$, $\underline{W_4W_4}$, $\underline{w_mw_m}$	G	magenta
L62-906	$\underline{w_1w_1}$, $\underline{w_3w_3}$, $\underline{W_4W_4}$, $\underline{W_mW_m}$	G	white

1 Pubescence codes: T=tawny, G=gray
2 Pedigrees: L70-4448 =L6(6) x PI 81.763
L6 = (Clark(8) x CNS) x (Clark(8) x Blackhawk)
L68-705 =L2(6) x PI 81.763
L2 = Harosoy 63 xL3
L3 = Harosoy(6) x S54-1207
S54-1207 = Hawkeye x (L49-4091 xL46-2132-1)
L49-4091 = F_3(Lincoln(2) x Richland) x F_1(Lincoln x CNS)
L46-2132-1 =Lincoln(2) x Richland

Table 2. Segregation in the F_2 population for each specified cross

Cross	Observed phenotypes1			Expected ratio	df	χ^2	P2
Harosoy $\underline{w_4}$ x Clark $\underline{w_4}$	130NWT	32NWG		3:1	1	2.379	0.123
Harosoy 63 x Clark $\underline{w_4}$	134P	50NW		3:1	1	0.464	0.496
L72-1078 x Clark $\underline{w_4}$	179DP	38NW		3:1	1	6.490	0.011
L72-1138 x Clark $\underline{w_4}$	121NWT	36NWG		3:1	1	0.359	0.549
T235 x Clark $\underline{w_4}$	120P	45NW	31M	9:4:3	3	2.088	0.352
L62-906 x Clark $\underline{w_4}$	131P	81W		9:7	3	2.646	0.104
Clark $\underline{w_4}$ x Harosoy 63	135P	48NW		3:1	1	0.148	0.701
Clark $\underline{w_4}$ xL72-1078	113DP	28NW		3:1	1	1.988	0.159

1 Flower color codes: NW=near white, W=white, P=purple, DP=dilute-purple, M=magenta
pubescence codes: T=tawny, G=gray
2 Probability of obtaining a greater value of chi-square by chance alone

Determination of dilute-purple, near-white, and Clark $\underline{w_x}$ near-white phenotypes was accomplished by dissection of mature flowers. Examination of the intensity of pigmentation in the veins or base of the flower petal and comparison with the parental isolines distinguished between the three phenotypes. The dilute-purple phenotype has distinct pigmentation confined to the throat region of the petal. The near-white phenotype displays faint pigmentation (if detected) in the veins and is usually concentrated in the throat area of the petal. The Clark $\underline{w_x}$ near-white phenotype has a hint of pigmentation in the veins of the petal, identical to the near-white phenotype described previously.

Results and Discussion

The data observed in the F_2 population conformed well to the expected segregation ratios of a single gene inheritance model. The cross Harosoy $\underline{w_x}$ x Clark $\underline{w_x}$ was made to test the homozygosity of the recessive $\underline{w_x}$ alleles in each parent. The data indicate that all the flower phenotypes of the F_2 progeny of this cross were the near-white color characteristic of the Clark $\underline{w_x}$ phenotype. This cross also allowed for linkage analysis between the $\underline{w_x}$ and the $\underline{T}$ locus encoding tawny pubescence. An observed 130 NWT (near-white, tawny) : 32 NWG (near-white, gray) fits the 3:1 expected ratio, indicating independence between $\underline{w_x}$ and $\underline{T}$.

An analysis of the progeny from the cross Harosoy 63 x Clark $\underline{w_x}$ indicates that the $\underline{w_x}$ locus is inherited in a 3 purple : 1 near-white ratio as expected for a single dominant gene (Table 2). The reciprocal cross (Clark $\underline{w_x}$ x Harosoy 63) confirms the fit of the expected model (Table 2) and confirms that $\underline{w_x}$ is a nuclear gene.

In the cross L72-1078 x Clark $\underline{w_x}$, independence was found between the $\underline{w_x}$ and $\underline{w_3}$ loci as indicated by the data fitting to a 3:1 model for single gene inheritance (Table 2). Deviations may have arisen due to an error associated with misclassification of some plants. The Harosoy $\underline{w_x}$ and Clark $\underline{w_x}$ plants produced flowers that were near-white with a slight purple color in the veins of the petals. In observing the F_2 progeny of this cross, the petals had to be removed and the throat region examined. Any indication of a purple color in this region may have been counted as dilute-purple instead of near-white. However, in the reciprocal cross (Clark $\underline{w_x}$ x L72-1078), the progeny segregated closer to the 3 dilute-purple : 1 near-white expected ratio (Table 2).

The cross L72-1138 x Clark $\underline{w_x}$ generated progeny that were difficult to classify.

The L72-1138 near-white flower phenotype and the Clark w_x near-white phenotype are identical in appearance. However, the segregation of pubescence types of the F_2 plants in this cross confirmed nonlinkage between the w_x locus and the T locus (Table 2).

The progeny of the cross T235 x Clark w_x allowed for examination of the inheritance and possible linkage of the w_x and w_m loci. The problem of phenotype misclassification error was avoided by combining the near-white and double recessive phenotypes into a single group. This modified the expected ratio from 9:3:3:1 to 9 purple : 4 near-white : 3 magenta. The data conform well to the expected model (Table 2). This would indicate independence between the w_x and the w_m loci.

The cross L62-906 x Clark w_x examined inheritance patterns associated with two loci, w_1 and w_x, with dominance at both loci. Again, the problem of phenotype misclassification was minimized by grouping all the near-white and white flower phenotypes into a single class. This altered the 9:3:3:1 expected ratio to a modified expected ratio of 9 purple : 7 white (includes near-white class). The observed data (131 purple : 81 white) fits the expected model, suggesting the w_x locus is independent of the w_1 locus.

Conclusion

In conclusion, the w_x locus acts epistatically with other loci controlling flower color to produce pigmentation in flowers of genotypes containing dominant W_1 and W_4 alleles. Data analysis indicates that the w_x locus does not appear to be linked to the w_1, w_3, or w_m loci. However, the w_x locus can be modified by the presence of dominant W_3 to confine the pigmentation to the throat of the flower. Controlled modification by the recessive w_m allele in the presence of W_1 and Wx will generate magenta pigmentation. Analysis of this data suggest that the novel source of w_x is at the same locus and apparently is the w_4 allele.

References

Bernard, R.L. 1974. Notice of release of Clark and Harosoy isolines. Soybean Genetics Newsletter 1:66-75.

Buzzell, R.I., B.R. Buttery, and R.L. Bernard. 1977. Inheritance and linkage of magenta flower color in soybeans. Can. J. Cytol. 19:749-751.

Groose, R.W. and R.G. Palmer. 1987. New mutations in a genetically unstable line of soybean. Soybean Genetics Newsletter 14:164-176.

Groose, R.W. and R.G. Palmer. 1991. Gene action governing anthocyanin pigmentation in soybean. J Heredity 82:498-501.

Groose, R.W., H.D. Weigelt, and R.G. Palmer. 1988. Somatic analysis of an unstable mutation for anthocyanin pigmentation in soybean. J Heredity 79:263-267.

Hartwig, E.E. and K. Hinson. 1962. Inheritance of flower color of soybeans. Crop Sci. 2:152-153.

Hedges, B.R. and R.G. Palmer. 1993. Mapping the w_4 locus in soybean. Soybean Genetics Newsletter 20:20-23.

Stephens, P.A. and C.D. Nickell. 1992. Inheritance of pink flower in soybean. Crop Sci. 32:1131-1132.

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Viruses Infecting Soybean in Tennessee

Soybean [Glycine max (L.) Merr.] is the leading field crop in terms of acreage and dollar value in Tennessee. Tennessee presently ranks fifteenth in the United States in soybean production (Brantner and Guinn, 1988); therefore diseases which affect yield are of considerable importance. Despite the importance of this crop, information on occurrence of virus diseases of soybeans in Tennessee has not been available.

Maximum yield losses of 10% to 100% have been attributed to virus diseases of soybeans Bean pod mottle virus (BPMV), bean yellow mosaic virus (BYMV), cowpea chlorotic mottle virus (CCMV), peanut mottle virus (PMV), peanut stunt virus (PSV), tobacco ringspot virus (TRSV), and soybean mosaic virus (SMV) have been reported infecting soybeans in states bordering Tennessee. Other viruses reported to infect soybeans in the United States are alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), southem bean mosaic virus (SBMV), tomato spotted wilt virus (TSWV), and white clover mosaic virus (WCMV) (Sinclair, 1982). AMV, BYMV, CMV, PSV, TRSV, and TSWV have been reported to infect other crops in Tennessee (McLaughlin, 1983; Milsaps et al., 1981; Reddick et al., 1987). In this paper we report the identification and relative occurrence over two years of viruses infecting soybeans in Tennessee. In order to ascertain if these soybean viruses were evenly distributed throughout the state, a strategy to sample 18 counties across three regions in Tennessee was adopted.

Materials and Methods

In 1987 and 1988, a survey was conducted in the state of Tennessee to detect the natural occurrence and distribution of twelve viruses infecting commercial soybeans. Plants in 2-5 soybean fields in each of 18 counties representing the major production areas of East, Middle, and West Tennessee were surveyed for virus infection. Six leaf samples were taken from each of the four soybean fields per county.

Five of these samples were leaves with virus-like symptoms such as mosaic, mottle or leaf distortion. The sixth sample was taken from a symptomless plant to use as a healthy comparison.

Viruses were identified by Protein-A sandwich enzyme-linked immunosorbent assay (PAS-EIISA) as described by Edwards and Cooper (1985). Protein A was used at a concentration of I ug/ml. Anfiserum for each virus (AMV, BPMV, BYW, CCMV, CMV, PMV, PSV, SBMV, SMV, TRSV, TSWV, and WCMV) was used at a 1:1,000 dilution. Protein-A alkaline phosphatase was used at a concentration of 0.4 lag/ml, and substrate (p-nitrophenylphosphate) was at I mg/ml. Samples to be tested were at a dilution of 1/20 (w/v). Duplicate wells of each sample were tested against all twelve antisera listed above. To compare data between regions a Fisher's Exact 2-aUed test was used to test the hypothesis of no difference in prevalence of a virus between regions.

Results

No data were taken on virus incidence; however, in most fields surveyed a visual estimate of between 1 and 5 % of the plants showed virus-like symptoms and no soybean field was observed in which more than 10% of the plants showed virus-like symptoms. All asymptomatic plants that were collected, tested negative for virus by PAS-ELISA.

In the 1987 survey, virus infected soybeans were found in 14 of the 18 counties. AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV, SMV, TRSV, and TSWV were detected (Table 1). Neither PMV nor WCMV were detected in any county. BPMV was the most prevalent virus detected. It was found in 50% of the fields tested (Table 1). All other viruses detected were found in less than 10% of the total fields tested, respectively (Table 1). Regional differences in occurrence of a virus, estimated by a Fisher's exact two tailed test, were significant only in the cases of BPMV and CCMV (Table 1). BPMV was found in all 8 counties and 82 % of the fields tested in West Tennessee, and in 2 of 7 counties and 25 % of the fields tested in Middle Tennessee (Table 1).

In 1988, either AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV, or TRSV were detected in all of the 18 counties surveyed (Table 1). PMV, SMV, TSWV, and WCMV were not detected in any county. Of the eight viruses found, in samples that tested positively for virus, BYMV was found in 31 % of the fields tested followed by BPMV in

27 %, TRSV in 21 %, CMV in 15 % and SBMV in 11 % of the fields tested (Table 1). Regional differences in occurrence of viruses were tested by a Fisher's exact 2-tailed test, and were significant in the cases of AMV, BPMV, BYMV, CMV, and SBMV (Table 1). BPMV was found in 40% of the fields tested in 7 of 8 counties in West Tennessee and in 23 % of the fields tested in only 2 of 7 counties in Middle Tennessee. BYMV was found in 60 % of the fields in 2 of 3 counties in East Tennessee, 45 % of the fields in 5 of 7 counties in Middle Tennessee and 10% of the fields in 2 of 8 counties in West Tennessee Crable 1). TRSV was the third most prevalent virus found in 1988. No significant regional differences in distribution of TRSV were found (Table 1). SBMV was found only in Middle Tennessee in 3 of 7 counties in 32% of the fields tested. CMV was found in 4 of 7 counties in 36% of the fields tested in Middle Tennessee (Table 1).

Discussion

This is the first report of BPMV in Tennessee. The source of initial virus inoculum is most likely infected soybean seed but, wild perennial hosts such as Desmodiiim paniculum (L.) D.C. may also be contributing to initial inoculum. BPMV was found in both years in primarily West and Middle Tennessee in areas that had large soybean acreage.

BYMV was the second most prevalent virus found. BYMV has been detected in halfrunner beans (Phaseolus vulgaris L.) in Middle Tennessee (Reddick, unpublished). Since BYMV has not been shown to be seed transmitted in soybeans, the presence of reservoir hosts for this virus in Tennessee indicates the potential for aphid vectors to spread this pathogen to soybeans in the field. This is the first report of BYMV infecting soybeans in Tennessee. BYMV was found primarily in East and Middle Tennessee in areas where other legumes such as snapbeans and clover are prevalent.

TRSV was the third most prevalent of the viruses detected in this survey. This virus has a wide host range, infects such crops as tobacco (Nicotiana tabacum L.) and cucumber (Cucumis sativas L.), and is seed transmitted in soybeans. Since an aerial vector for TRSV is not known at this time, the initial source for infection in soybeans is most likely infected seed. This is the first report of TRSV infecting soybeans in Tennessee.

Table 1. Distribution of viruses detected by PAS-ELISA in plants with virus symptoms in the 1987 & 1988 soybean virus survey.

County	Fields Sampled		No. Fields with Soybean Plants Testing Positive for Virus																			
			AMV*		BPMV*		BYMV*		CCMV*		CMV*		PSV		SBMV*		SMV		TRSV		TSWV	
	87	88	87	88	87	88	87	88	87	88	87	88	87	88	87	88	87	8	87	88	87	88
East Tennessee																						
Blount	3	2		2								1										
Monroe	4	4	1					2	2		1		1				1					
Fentress	4	4					1	4												1		
Total Fields	11	10	1	2	0	0	1	6	2	0	1	1	1	0	0	0	1	0	0	1	0	0
Middle Tennessee																						
Bedford	2	4		1		3		3				4				3				1		
Giles	3	4		1	1					1				1						2		
Hickman	2	2						2				1				2				2		
Humphreys	3	4						1				2				2						
Maury	2	2									1	1		1								
Robertson	5	3			4	2	1	2														
Rutherford	3	3						2	1				2							1		
Total Fields	20	22	0	2	5	5	1	10	1	1	1	8	2	2	0	7	0	0	0	6	0	0
West Tennessee																						
Dyer	4	4			4	3					1		1		1					2		
Gibson	4	4			3	1													2			
Haywood	4	4			1	2														1		
Lake	4	4			4	2									1				2	2	1	
Lauderdale	4	4			4															1	1	
Madison	5	4			3	1																
Obion	4	2			4	1							2		2				1			
Tipton	4	4			4	2	1						2		2				1			
Total Fields	33	30	0	0	27	12	1	0	0	0	1	0	3	0	4	0	0	0	5	6	2	0

NOTE: "*" indicates a significant difference among regions among regions at P=0.01 when analyzed by Fisher's Exact Two-Tailed test.

AMV, CCMV, CMV, PSV, SBMV, SMV, and TSWV were detected in low frequencies throughout the 2 year survey (Table 1). It is surprising that SMV was detected in only one sample from Monroe county. SMV, an aphid transmitted and seed-bome virus, has been reported as a prevalent virus throughout the major soybean growing areas of the southem United States. Because SMV does not occur naturally in species other than G. max the primary source of inoculum in the field is infected seed. This is the first report of SMV in Tennessee.

TSWV-type, a thrips-transmitted virus, was detected in Lake and Lauderdale counties in West Tennessee in 1987 (Table 1). This virus was also identified in three other plants from soybean cultivar field tests in East and West Tennessee (data not shown). This is the first report of its natural occurrence in soybeans and is of interest since this virus has recently been detected in tobacco, tomato and pepper in Tennessee. Importance of this virus in soybeans has not been determined.

PMV and WCMV were not detected in any county during the two years surveyed. Although disease incidence was not calculated in individual fields, virus incidence appeared low since it was sometimes difficult to collect five symptomatic samples from the majority of the counties in Middle and East Tennessee. BPMV, BYMV and TRSV were the most prevalent viruses found in this survey. BPMV was found primarily in West Tennessee, whereas BYMV was found primarily in East and Middle Tennessee. TRSV was evenly distributed throughout the state. Tennessee does not appear to have a very severe virus disease problem in soybeans at this time, but the potential for virus epidemic is present.

References

Branter, R., and B. Guinn. 1988. Tennessee agricultural statistics. Tennessee Dept. Agric. Bull. 325809. 131 pp.

Edwards, M.L., and J.I. Cooper. 1985. Plant virus detection using a new form of indirect, ELISA. J. of Virol. Methods 11:309-319.

McLaughlin, M.R. 1983. Viruses infecting forage legumes in Tennessee. Plant Dis. 67:490-492.

Mfllsap, D.S., M.R. McLaughlin, C.H. Hadden, and P.P. Hunter. 1981. Virus diseases of burley tobacco in Tennessee. (Abstr.) Phytopathology 71:895.

Reddick, B.B., C.H. Hadden, S.C. Bost, and M.A. Newman. 1987. First report of tomato spotted wilt virus in Tennessee. Plant Dis. 71:376.

Sinclair, J.B. 1982. Virus diseases. pp 51-59 in: Compendium of Soybean Diseases. The Am. Phytopathol. Soc., St. Paul, Minnesota.

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Inheritance of brown stem rot resistance in soybean isolines L68-0327 and L68-0469 and germplasm lines L84-5873 and L84-5932

Brown stem rot of soybean, causal agent Phialophora gregata, is the most frequently observed soil borne disease in Illinois (Eathington, 1992). Several cultivars have been developed and released containing brown stem rot resistance derived from P.I. 84946-2. Breeders have also developed germplasm lines that contain brown stem rot resistant genes from other sources and isolines that contain brown stem rot resistant genes from P.I. 84946-2. If soybean breeders are to efficiently use these lines, it would be helpful to know which resistance genes they contain. Our objective was to study the inheritance of brown stem rot resistance in the isolines L68-0327 and L68-0469, and the germplasm lines L84-5873 and L84-5932.

Materials and Methods

The two brown stem rot resistant isolines were developed using Harosoy and Clark as recurrent parents and P.I. 84946-2 as the source of brown stem rot resistance. The brown stem rot resistance in the Clark isoline and 'BSR 101' can be traced to L59-1891A-5, whose parentage is Clark (3) x P.I. 84946-2 (R. L. Bernard, personal communication). The two brown stem rot resistant germplasm lines (L84-5873 and L84-5932) derive their resistance from P.I. 86150 and P.I. 90138 (Nickell and Bernard, 1992).

We crossed the isolines and germplasm lines used in this study with soybean lines carrying the three known brown stem rot resistant genes [L78-4094(Rbs_1), P.I. 437.833 (Rbs_2), and P.I. 437.970 (Rbs_3)], resulting in 12 populations. Thirty-nine to fifty $F_{2:3}$ families derived from each population, parents, and susceptible cultivars were classified for their reaction to a Type I isolate of P. gregata (Oh2) by classifying two

replicates, each containing a single pot with five plants from each family. We used a root-dip inoculation technique that differentiated the resistant and susceptible check cultivars (Sebastian et al., 1983). We classified the lines as resistant or susceptible by analyzing both leaf and stem symptoms of the checks, thus determining the leaf and stem symptom percentages that classified the resistant and susceptible checks most accurately within each replicate. We classified a family only if it contained seven or more plants. We designated families as resistant if they had one or zero susceptible plants, heterogeneous if they had two or more susceptible plants, and susceptible if most of the plants were susceptible. There is a 20% probability of classifying a heterogeneous family segregating 3:1 as a resistant family when classifying a minimum of seven plants and designating families with one susceptible plant as resistant.

We used Chi-square analysis to test the populations for goodness of fit with both two and three gene models of inheritance for the brown stem rot resistance genes. Because of the inability to identify heterozygous families segregating at 15:1 and 63:1 ratios, we combined these heterozygous families with the families that are all resistant.

Results and Discussion

Brown stem rot leaf and stem symptoms were readily observed in the greenhouse. L84-5932 had the highest level of resistance with a 0% leaf and a 1% stem symptom average.

Harosoy Isoline - L68-0327

There were no susceptible F_2 plants in the population created by crossing L68-0327 with L78-4094 suggesting that L68-0327 may contain $\underline{Rbs_1}$ (Table 1). However, segregation of the $F_{2:3}$ families and F_3 plants fit a two gene model, indicating that L68-0327 contains a single gene for resistance, which is not allelic to $\underline{Rbs_1}$. The F_2, $F_{2:3}$, and F_3 data from the cross of L68-0327 with P.I. 437833 all fit a two gene segregation model, confirming that there is a single gene for resistance in L68-0327, and that it is not allelic to $\underline{Rbs_2}$. There were too many susceptible F_2 plants from the cross of L68-0327 with P.I. 437970 to fit a 15:1 ratio, suggesting that L68-0327 does not contain $\underline{Rbs_3}$. $F_{2:3}$ family data fit a two gene model; however, the F_3 plant data did not fit this

model. Therefore, it is not clear from the segregation analysis if L68-0327 contains $\underline{Rbs_1}$, $\underline{Rbs_3}$ or neither. By examining distributions of leaf and stem symptoms of F_3 plants from the three populations involving L68-0327 it appears from leaf symptoms that L68-0327 contains $\underline{Rbs_3}$ (Figure 1). Stem symptoms do not help in suggesting one gene over the others. It was also observed that L68-0327 contains Rmd that imparts an adult-plant resistant reaction to powdery mildew as opposed to the susceptible reaction of Harosoy. Since in a previous study (Lohnes and Nickell, 1994) there were differences in the brown stem rot reaction among powdery mildew isolines, we believe a linkage exists and should be studied.

Clark Isoline - L68-0469

F_2 plant data from the populations created by crossing the Clark isoline, L68-0469, to L78-4094, P.I. 437833 and P.I. 437970 indicate that L68-0469 contains $\underline{Rbs_3}$. The $F_{2:3}$ family data agree with this because they fit a two gene model for L78-4094 and P.I. 437833 populations, but not with the P.I. 437970 population. The F_3 plant data only fit a two gene model for the L78-4094 population; however, the chi-square probabilities indicate that L68-0469 has $\underline{Rbs_3}$. Analysis of distributions of the leaf and stem symptoms also suggest that L68-0327 contains $\underline{Rbs_3}$ (Figure 2). The distribution of stem symptoms from the cross of L68-0469 x P.I. 437970 displays a slight bi-modal distribution; however, the leaf symptoms are clearly not distributed in a bi-modal manner, suggesting that L68-0469 most likely contains $\underline{Rbs_3}$. This result agrees with Eathington (1992) who found that the related cultivar, BSR 101, also contains $\underline{Rbs_3}$.

PI 86.150 Germplasm Line - L84-5873

The F_2 data from the populations created by crossing L84-5873 with L78-4094, P.I. 437833, and P.I. 437970 suggests that L84-5873 contains $\underline{Rbs_1}$. The L84-5873 x L78-4094 population contains only one susceptible plant, while the other populations had too many susceptible plants to fit a 15:1 ratio. The $F_{2:3}$ family data does not concur with the F_2 plant data in that the populations from crosses of L84-5873 with L78-4094 and P.I. 437833 fit a 2 gene segregation model, and the P.I. 437970 population does not, suggesting that L84-5873 contains $\underline{Rbs_3}$. The F_3 data had too few susceptible plants to fit a two gene model for any of the populations, however the F_3 data indicate that L84-5873 most likely contains $\underline{Rbs_3}$. The distribution of leaf symptoms of F_3 plants suggests that L84-5873 contains $\underline{Rbs_3}$, however the distribution

of stem symptoms is not as conclusive (Figure 3). From stem symptoms it appears that L84-5873 does not contain $\underline{Rbs_1}$, however, it is not clear if there is segregation with $\underline{Rbs_2}$ and $\underline{Rbs_3}$.

Overall, the analysis suggests that L84-5873 contains $\underline{Rbs_3}$. This study agrees with Willmot et al. (1988) who found that P.I. 86150 had a single gene for resistance to brown stem rot. However, this disagrees with Willmot et al. (1988) who found that the brown stem rot resistance gene in P.I. 86150 is not allelic to any of the brown stem rot resistance genes in P.I. 84946-2. Since two lines which derive their brown stem rot resistance from P.I. 84946-2 (BSR 101 and L68-0469) contain $\underline{Rbs_3}$, it is apparent that P.I. 84946-2 contains both $\underline{Rbs_1}$ and $\underline{Rbs_3}$.

I 90.138 Germplasm Line - L84-5932

F_2 plant data from the populations created by crossing L84-5932 with L78-4094, P.I. 437833 and P.I. 437970 indicate that L84-5932 contains two genes for resistance to brown stem rot of which one is $\underline{Rbs_1}$. There were no susceptible plants in the L78-4094 population, but some in the other populations. The $F_{2:3}$ family data agree with this because it fits a three gene model for both populations from the crosses of L84-5932 with L78-4094 and P.I. 437833; however, the P.I. 437970 population also fit, but only because it had one heterogeneous family. The F_3 plant data fits a three gene model for the P.I. 437833 and P.I. 437970 populations, but not the L78-4094 population, indicating that L84-5932 contains two resistance genes of which one is $\underline{Rbs_1}$. The distribution of leaf and stem symptoms of F_3 plants is inconclusive (Figure 4). Overall, it appears there are two brown stem rot resistant genes in L84-5932, one of these is $\underline{Rbs_1}$, and the other appears to be located at an unidentified locus.

In conclusion, it appears that the P.I. 90138 germplasm line, L84-5932, is a good source of brown stem rot resistance for developing resistant cultivars. It has the highest level of resistance of any of the resistant cultivars tested, and it has two genes for resistance that have not been used in the development of public soybean cultivars. L84-5873 also had a high level of resistance, but it appears to contain the resistance gene that has already been used to develop brown stem rot resistant cultivars.

References

Eathington, S.R. 1992. Soybean disease pressure in Illinois and the inheritance of

brown stem rot resistance. MS Thesis., Univ. of Illinois, Urbana. p. 108.

Lohnes, D.G. and C.D. Nickell. 1994. Effects of the powdery mildew alleles Rmd-$_c$, Rmd, and rmd on yield and other characteristics in soybean. Plant Disease 78:299-301.

Nickell, C.D. and R.L. Bernard. 1992. Registration of L84-5873 and L84-5932 soybean germplasm lines resistant to brown stem rot. Crop Sci. 32:835.

Sebastian, S.A., C.D. Nickell, and L.E. Gray. 1983. Sequential screening of soybean plants for resistance to Phytophthora rot and brown stem rot. Crop Sci. 23:1214-1215.

Willmot, D.B., C.D. Nickell, and R.L. Nelson. 1988. Preliminary study on the inheritance of brown stem rot resistance in P.I. 86150 and P.I. 423930A. Soybean Genet. Newsl. 15:103-106.

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Genetic and genotype x environment effects of Mexican bean beetle defoliation on vegetable-type soybean genotypes

Consumers across the United States, concerned with their health and physical fitness, are seeking alternative vegetable crops that can be incorporated into a low calorie diet. Vegetable-type soybean [Glycine max (L.) Merr] cultivars have shown promise as a new crop that can be incorporated into low calorie diet. These specialty soybean cultivars have flavor, texture, and appearance characteristics that often differ from cultivars grown for edible vegetable oil or for high protein feed supplements for livestock (Morse, 1950).

Soybeans in general are limited in reaching their maximum yield potential due to environmental and biological stress factors such as Mexican bean beetle (MBB). The MBB, Epilachna varivestis Mulsant, is an important defoliator of soybean in the eastern United States and portions of the Midwest (Mellors et al., 1984). Although insecticides provide adequate control of MBB and other insects, the use of host plant resistance to control these insects would be an economically and ecologically superior management technique (Sisson et al., 1976).

Progress in breeding for economic characters that are quantitative in inheritance is subject to environmental variability and is determined by the nature and magnitude of the genetic variability. Such characters present difficulty in selection programs, because heritable variation is masked by non-heritable components. Hence, there is a need to partition the overall variability into heritable and non-heritable components with the aid of genetic parameters such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variations (PCV) and heritability. The objectives of this study were: 1) to determine the magnitude of Genotype x year interaction and 2) to estimate the phenotypic and genotypic variability and heritability

Material and Methods

This study included of 129 vegetable-type soybean genotypes, 22 grain-type,

six MBB-susceptible and sixteen MBB-resistant genotypes. The vegetable-type genotypes were selected based on seed size (<10g/100 or > 20g/100 seeds). A total of 173 selected soybean genotypes were planted in 2-m rows, 25 seeds/m, with 1-m spacings, and 0.9-m width between rows. A mixture of bush lima beans, Phaseolus vulgaris L. and snap beans, Phaseolus lunatus L., was planted in every third furrow 3 weeks before the soybeans were planted. The snap and bush beans attracted a local population of MBB just emerging from hibernation, and provided for their population growth. In addition, several thousand hatched egg masses from greenhouse-reared MBB were transferred to the bean foliage in the experimental plots. A microclimate favorable to MBB development was maintained by using overhead irrigation system during periods of water stress.

Each maturity group (MG) was planted in a separate block to allow comparisons within the same growth stage. The earlier maturity groups were planted later to allow MBB populations time to develop before plant maturity; i.e., MG VII and VIII were planted in early June, MGs V and VI in mid June, and MGs III and IV in late June. Defoliation was determined visually by estimating the percentage, in increments of 5%, of total leaf area in each 2-m row that had been consumed by MBB.
The experimental design for each MG was a randomized complete block (RCBD) with three replications. The data of each MG were analyzed as RCBD combined over years. Variance components were calculated by equating appropriate mean squares to their expectations and solving for the components. Broad sense heritability estimates (H_{BS}) using variance components from mixed model analysis were calculated as described by Milligen et al., (1990): $H^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{gy}/y + \sigma^2_e/ry)$. The terms σ^2_g, σ^2_{gy}, and σ^2_e refer to the estimates of genotypes, genotype x environment interaction,and error variance, respectively. The divisor y refers to number of years and n number of replications per year. The variance components were used to compute the phenotypic coefficient of variability (PCV), the genotypic coefficient of variability (GCV), and heritability (in the broad sense). The PCV and GCV were calculated according to the methods of Kumar et al. (1985): $PCV = 100\ (\sigma^2_{ph})^{1/2}/\bar{x}$; $GCV = 100\ (\sigma^2_g)^{1/2}/\bar{x}$. Where σ^2_g and σ^2_{ph} are estimates of genotypic and phenotypic variances and $\bar{x}$ is the mean of the MBB foliar damage.

Results and Discussion

Significant year-to-year variations were observed in the analysis of variance for

the data combined over years for each MG. The genotypic variations were significant for all MGs which indicates genetic variation exist for selection and hybridization. A significant genotype x year interactions were present for all MGs. These interactions were the result of a change in the magnitude of differences between the genotypes in different years. The significant interactions observed suggest that the performance or response of the genetic materials used in this study were not stable from one season (year) to another. This is not surprising considering the range of environments encountered; however, σ^2_g was larger than the σ^2_{gy} for each MG tested except MG III (Table 1). This indicates that genetic variation for resistance to MBB foliar feeding exists among the genotypes and that selection for this trait should be effective.

Table 1. Variance components, standard errors, mean, heritability, genotypic and phenotypic coefficient of variations.

	Maturity Group					
Parameter	III	IV	V	VI	VII	VIII
σ^2_g	23.4	48.1	106.0	102.0	99.1	6.4
± S.E.	15.5	14.5	29.8	32.5	38.5	26.5
σ^2_{gy}	70.6	40.8	78.8	44.0	78.2	33.6
± S.E.	8.2	7.8	4.7	11.0	18.8	3.7
σ^2_e	36.4	23.9	42.8	38.5	29.6	37.9
± S.E.	4.3	2.2	3.8	4.3	3.7	5.3
Mean	25.2	24.4	24.8	21.4	18.0	17.8
± S.E.	2.0	1.6	2.2	2.1	1.8	2.1
H^2	45.9	74.7	77.3	84.3	77.1	80.9
± S.E.	1.1	1.0	1.0	1.1	1.0	1.0
GCV	19.2	28.5	41.4	47.3	55.2	45.5
PCV	28.3	32.9	47.2	51.5	62.8	50.6

Comparing the relative contribution of genotypic variance to total component variances, we found that MG VI had the highest (55%), followed by MGs VII and VIII both with 48%. Maturity group III had the lowest with only 18%. On the other hand the

relative contribution of the $\sigma^2_{g \times y}$ interaction for MG VII was 54% and MG VIII had the lowest 34%. The relatively error magnitude ranked in decreasing order was MGs VIII, VI, III, V, VII and IV. The error variance proportional contribution to the total variance was as high as 28% for MG III and as low as 14% for MG VII.

The genotypic coefficient of variation (GCV) values ranged from 19.1 to 55.17 (Table 1). The late MGs V, VI, VII, and VIII had high GCV values which were over 42% and the early MGs III and IV had 19% and 28%, respectively. The PCV values showed the same trend as the GCV. The PCV values ranged from 28% for MG III to 63% for MG VII. Again high PCV values were observed in the late MGs V, VI, VIII, and VIII and the early MGs III and IV showed the lowest. The heritability estimates ranged from 46% to 84%. Maturity groups VI had the highest heritability estimate and MG III had the lowest. These high heritability estimate values are encouraging, because these imply that selection for improved MBB foliar feeding resistance can be achieved in breeding populations established from this soybean germplasm. Almost all the MGs showed high heritability estimates for MBB foliar feeding with the exception of MG III. The low heritability estimates observed in MG III suggested that selection for MBB defoliation would be relatively difficult.

References

Kumar, A.S.C. Misra, Y. P. and B.P.S. Singh. 1985. Variability and correlation studies on triticale. J. Moharashtra Agricultural University 10:273-275.

Mellors, W.K., A. Allegro, and S.E. Propts. 1984. Adult reproductive diapause in the Mexican bean beetle (Coleoptera:Coccinellidae): Interaction of temperature with photoperiod. Environ. Entomol. 13:409-414.

Sisson, V.A., P.A. Miller, W.V. Compbell, and J.W. Van Duyn. 1976. Evidence if inheritance of resistance to the Mexican bean beetle in soybeans. Crop Sci. 16:835-837.

Milligan, S.B. K.A. Gravois, K.D. Bischoff, and R.A. Martin. 1990. Crop Effects on broad sense heritability and genetic variances of sugar cane yield components Crop Sci. 30:344-349.

Morse, W.J. 1950. Chemical composition of soybean seed, p. 22-150. In: I. S. Markley (ed.) Soybean Products. John Wiley and Sons, New York.

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Investigation of Lectin Nulls in Perennial Glycine Species Introduction

Lectins are a group of plant proteins which are distinguished by their ability to bind specific sugar molecules. Le_1 is the single gene that produces lectin protein in Glycine max (Orf et al., 1978, Vodkin et al., 1983). Nulls for lectin protein in the subgenus Glycine have been identified but there has not been any molecular biological study of these nulls (Garcia-Orbegozo, 1990). The investigation sought to achieve two objectives. The first was to amplify the lectin gene in perennial Glycine species that was most homologous to the Le_1 gene in soybean G. max This was done by constructing primers to conserved sites on the Le_1 gene of soybean. The conserved sites were found by comparing the soybean gene sequence in six species including soybean. The second objective was to explore the question: "Is the lectin null in perennial Glycine species due to an insertion in the Le_1 gene as found previously?" (Vodkin et al., 1983)

Materials and Methods

Lectin positive and null seeds from three wild diploid Glycine (2n = 40) species, [G. canescens (CAN), G. clandestina (CLA), and G. tabacina (TAB)], were grown in the greenhouse. DNA was extracted from the leaves using two methods; cesium chloride extraction and the mini prep DNA extraction method. PCR was performed on all of the samples using the following program: A 2 min denaturation step at 96 °C; followed by 40 cycles of 20 sec denaturation at 96 °C, a 1 min annealing step at 36 °C, a 2 min synthesis at 72 °C, and a 7 min extension step at 72° The primers used for PCR were designed from the sequence of Le_1 (Vodkin et al., 1983). The Le_2 sequence (Vodkin et al., unpublished) was used to compare with the Le_1 sequence when constructing primers.

Table 1. The DNA samples of Glycine species used in this study with concentration and presence (+) or absence (-) of lectin.

Sample number	Accession number G. soja	Lectin
073	So407.184-3A	-
061	So339.732-6	-
049	So407.217-1	-
051	So407.218-1	-
052	So407.252-2	-
065	So378.700-1	+
069	So366.120-2	+
071	So378.683-2	+
057	So339.731-4A	+
064	So65.549-2	+
053	So407.179-3	+
Sooty		-
Williams c278		+

Table 2. DNA samples of Glycine perennial species used in this study with concentration and presence or absence of lectin.

Species	Code	Accession number[a]		Lectin[b]
		IL	P.I.	
G. canescens	CAN	379	399478	+
G. clandestina	CLA	301	233138	-
G. clandestina	CLA	415	440945	-
G. clandestina	CLA	445	440955	-
G. clandestina	CLA	525	440970	+
G. tabacina	TAB	346	373992	+
G. tabacina	TAB	479	440987	+
G. tabacina	TAB	624	483198	+

[a]IL = Temporary number assigned at the University of Illinois, PI = Plant introduction number

[b]Presence (+) or absence (-) of lectin in the seed

Results and Discussion

In order to study the lectin gene, primers were constructed to conserved regions of the gene. These regions were found through a comparison of the lectin gene sequence between six different species. These species are: Medicago, Pisum sativum, Glycine max (Le_2, Le_1), Phaseolus vulgaris, Dolichos biflorus, and Dolichos vulgaris. Lec31 and Lec 32R were the first set of primers used to perform PCR (Figure 1). The Lec31 and Lec32R sequences are almost identical in the soybean Le_1 and Le_2 genes. Figure 2 shows PCR products from perennial Glycine species along with soybean using Lec31 and Lec32R primers. The fragment size expected is 529 bp (Figure 1). The gel picture shows fragments from wild Glycine species which are both + and - for lectin. There is no distinction between the + and - samples.

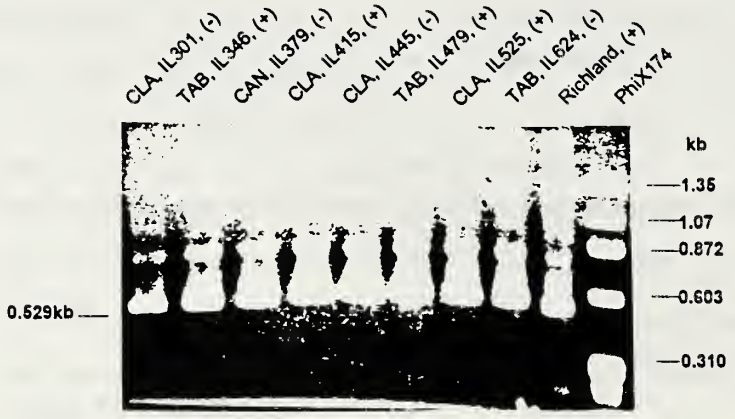

Fig. 2. PCR products from *Glycine* species run on a 0.7% agarose gel and stained with ethidium bromide. PhiX174 is a DNA molecular weight marker. All of the sample are run with primer set Lec31/32R. Samples are identified with the species code, accession number (see table 2 for details), and lectin presence (+) or lectin absence (-).

Lec31
5' CCCCCATCCACATTTGGGACA 3'

Lec32R
5' TTGGAAGCAAAAGACCAAGA 3'

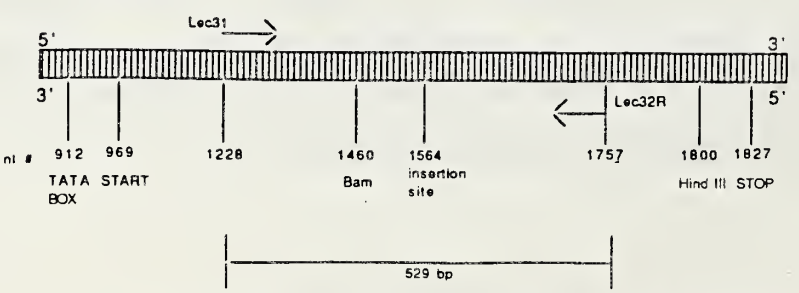

Fig. 1. A diagram showing the location of the Lec31/32R primers relative to the lectin gene.

A new set of primers were constructed in order to amplify only the Le_1 gene. The Lec33 shomologous region of the Le_1 gene. The same is true for Lec34R. The expected fragment size is 380 bp (Figure 3). Figure 4 shows the amplified PCR products from soybean cultivars Sooty (lectin-) and Williams (lectin +) using the primers Lec31/32R and Lec33/34R. The Lec31/32R set shows that both Le_1 and Le_2 were being amplified, because Sooty (-) has a band the same size as Williams (+). Since the Sooty lectin null is caused by the insertion of a transposable element in the Le_1 gene, the expected band size for Sooty would be approximately 3 kb. In contrast, the Lec33/34R primer set amplified only the Le_1 gene. This is shown by the lack of the 380 bp band in the Sooty lane. The lack of a fragment is due to the 3 kb insertion in the lectin gene. (Vodkin et al., 1983) The Williams lane shows the expected 380 bp fragment. These reactions were carried out in order to isolate a primer set specific for the homologous Le_1 gene in perennial Glycine. A Southern blot was performed on the gel in Figure 4 to confirm that all of the bands corresponded to the lectin gene. The probe hybridized to all of the expected bands. Figure 5 consists of lectin +/- wild perennial and G.soja samples run with primer sets Lec31/32R and Lec33/34R. There is no distinction between lectin + or lectin - samples.

Lec33
5' CTTTCCGGAACTCTTGGGATC 3'

Lec34R
5' GATGGCCTCATGCAACACAAA 3'

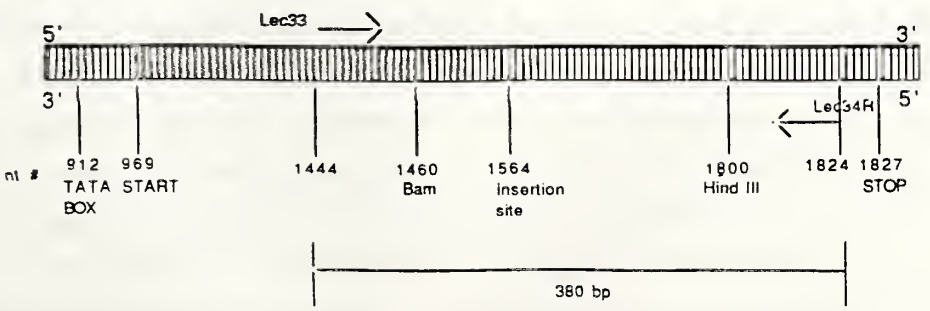

Fig.3. A diagram showing the location of the Lec33/34R primers relative to the lectin gene.

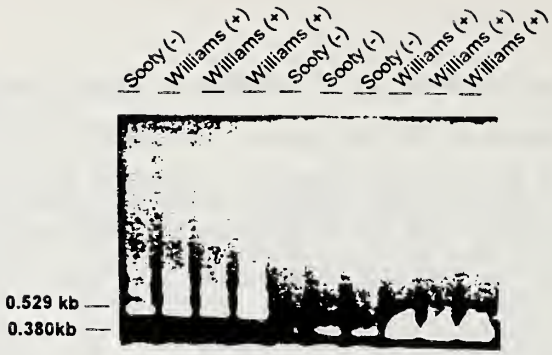

Fig.4. PCR products from *Glycine max* species run on a 0.7.% agarose gel stained with ethidium bromide. Samples 1-4 are run with primer set Lec31/Lec32R and samples 5-10 are run with primer set Lec33/34R. Samples are identified with variety name and lectin presence (+) or lectin absence(-).

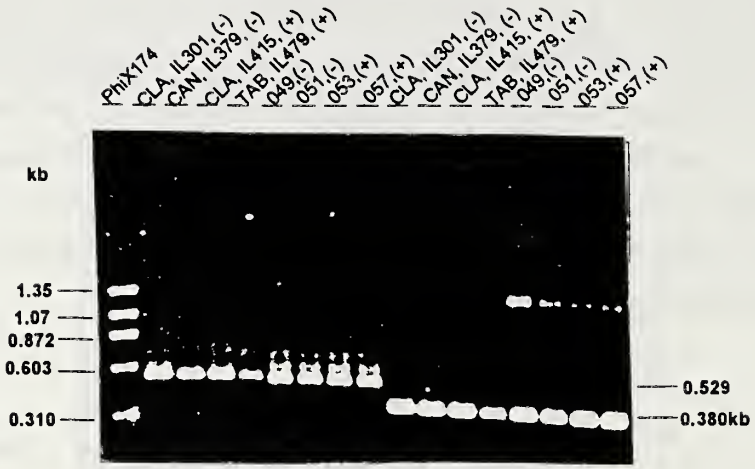

Fig.5. PCR products from *Glycine* species run on a 0.7% agarose gel and stained with ethidium bromide. PhiX174 is the DNA molecular weight marker. Samples 2-9 are run with primer set Lec31/Lec32R. Samples 1 0-17 are run with primer set Lec33/34R. Samples are identified with the species code, accession number (see table 1&2 for details), and lectin presence (+) or lectin absence (-).

Conclusion

In summary, Figure 5 containing Glycine species that are both lectin + and lectin -, does not show a difference in size of the amplified gene product. Therefore, the tentative conclusion is that an insertion element in the region between the primers is not the cause of the lectin null. However it is possible that the perennial Glycine species contains duplicated genes with sequence homology to the Le_1 gene that would produce a positive PCR reaction in all samples. The PCR reaction could be amplifying these duplicated genes. Additional research involving the sequencing and characterization of lectin genes in perennial Glycine species could reveal a mechanism for the lectin null.

References

Garcia-Orbegozo, E. 1990. The development of triple nulls for several seed proteins in Glycine max and Studies of seed lectin in the subgenus glycine. M.S. Thesis, University of Illinois, Urbana-Champaign, IL.

Orf, J.H., T. Hymowitz, S.P. Pull and S.G. Pueppke. 1978. Inheritance of a soybean seed lectin. Crop Sci. 18:899-900.

Vodkin, L.O., P.R. Rhodes and R.B. Goldberg. 1983. A Lectin gene insertion has the structural features of a transposable element. Cell 34:1023-1031.

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Phytate accumulation in vegetable-type soybean harvested at three reproductive stages

Consumers across the United States concerned with health and physical fitness are exploring alternative vegetable crops that can be incorporated into a low calorie nutritional diet. Vegetable-type soybean is very popular as a food in the Orient, where the incidence of heart disease is low. Vegetable-type soybean is also different from the mature soybean in flavor and texture (Morse, 1950) and is low in trypsin inhibitor (Gupta and Deodhar, 1975).

Soybean seed composition changes during development. Phytatic acid (PA), myo-inositol 1, 2, 3, 4, 5, 6 hexphosphate, is the storage form of phosphorous in soybean. The PA binds with nutritionally-important metals, especially zinc (Zn), calcium (Ca), manganese (Mn), magnesium (Mg), forming a phytate-metal complex that possibly contributes to nutritional deficiencies in non-ruminant animals. The growing interest in soybean as a source of protein has spurred an interest in its PA contents (Roboy et al., 1984; Reddy et al., 1982; Mohamed et al., 1986). In earlier report, we documented the existence of wide variation in PA among soybean genotypes harvested at R_8 (Mohamed et al., 1991). A breeding program aimed at reducing PA in soybean genotypes is in progress at Virginia State University (VSU). The major thrusts of the current plant breeding research at VSU is the development of vegetable type soybean cultivars with desirable agronomic traits and high nutritional quality and quantity. However, information on nutritional and anti-nutritional factors of vegetable-type soybean is needed to design an effective breeding program. The main objective of this study is to determine the phytate accumulation in selected vegetable-type soybean genotypes.

Materials and Methods

Seventeen vegetable-type soybean genotypes, six from each maturity group (MG) IV and V, and five from MG VI were selected on the basis of seed size, MG, and

seed availability. Three replications of each entry were planted in four-row plots, arranged in randomized complete block design, at Randolph Research Farm of VSU, Petersburg, Virginia. Each four-row plot was 4 m long and 3.60 m wide, with the spacing of 0.90 m between rows; a seeding rate of 23 seeds per meter was used. Each genotype was evaluated at three reproductive stages (R_5, R_6, and R_7, Fehr et al., 1971) for anti-nutritional factors by harvesting whole plants from one meter long of the two center rows of each plot. The harvested materials were immediately put in plastic bags and brought to the laboratory. Pods were removed from the harvested plants by hands. Green immature seeds were separated from the pods, freeze dried and ground. The samples were analyzed for phytate (PA) according to the procedures described Mohamed et al., (1986). Phytic acid was extracted by 3 % trichloroacetic acid solution (TCA). Phytate was purified on an anion exchange resin (Dowex-1X8CL$^-$) to remove inorganic phosphate, di, tri, and tetra phosphoinositol. The phytate was eluted from the resin using 1 M NaCl. The eluent was used to determine PA as described by Mohamed et al., (1986). Data were statistically analyzed and means were separated, by using Least Significant Difference (LSD) test at the 5% level of significance.

Results and Discussions

The mean data of PA content (mg/gm meal) of vegetable-type soybean genotypes harvested at three reproductive stages are presented in Table 1. Significant genotypic differences were observed for phytate among the tested genotypes. The genotype x stage interaction was also significant. These significant differences observed in this study indicated that genetic variation exist among genotypes tested for further selection and improvement. No significant difference for PA was found between seed sizes or MGs. These data are in agreement with the previous report by Mohamed et al., (1991).

The mean phytate of genotypes harvested at the three reproductive stages ranged from 6.3 to 26.0 (Table 1). The genotypes harvested at R_7 stage had the highest phytate mean (26.0) and the R_5 had the lowest (6.3). The variability in phytate content among mature soybean genotypes has been reported by Mohamed et al., (1986). The role of PA in human nutrition is documented by Erdman and Weingartner (1981); Synder and Kwone (1987) , and Hafez et al., (1989).

Table 1. Mean phytate content (mg/gm meal) of Vegetable-type Soybean Harvested at Three Reproductive Stages

Genotype	MG	Seed Size	Reproductive Stages R_5	R_6	R_7	Overall Mean
Ware	IV	Lg	8.2	15.6	25.7	16.5
Emperor	IV	Lg	5.4	11.5	18.6	11.8
Sango	IV	Lg	3.6	17.8	30.9	17.4
Kingston	IV	Sm	5.4	22.8	31.0	19.7
Sooty	IV	Sm	6.2	15.1	27.3	16.2
Wilson-5	IV	Sm	7.4	21.0	23.5	17.3
PI 416982	V	Lg	4.7	14.6	20.1	13.1
PI 417288	V	Lg	7.5	15.3	28.2	17.0
PI 417322	V	Lg	8.1	15.5	28.5	17.4
PI 416771	V	Sm	3.6	14.2	17.4	11.7
PI 417052	V	Sm	9.3	15.9	31.2	18.8
PI 423759	V	Sm	7.4	15.2	20.5	14.4
PI 417213	VI	Lg	6.0	11.6	26.3	14.6
PI 417310	VI	Lg	5.7	20.9	32.8	19.8
PI 423852	VI	Sm	5.6	16.9	29.5	17.3
PI 222397	VI	Sm	7.3	17.9	30.0	18.4
PI 171437	VI	Sm	5.2	17.2	28.0	10.8
Mean			6.3	16.5	26.0	15.8
CV %			13.3	11.0	7.17	17.7
* G. LSD (0.05)			0.96	2.15	2.34	1.7
** G x S LSD				1.53		

* G = Genotype
** G x S = Genotype x Growth stage

The overall genotypic mean was 15.8 and ranged from 10.8 to 19.8. The genotypes with low mean phytate values were P.I. 416771, 'Emperor', and P.I. 416982. Conversely, P.I. 417310, 'Kingston', and P.I. 417052 had the highest phytate values. A comparison of genotypes at each harvested stage indicated that P.I. 416771, 'Sango', Emperor and P.I. 416982 had low mean phytate content at R_5. The genotypes P.I. 416771, P.I. 416982 and Emperor that registered low phytate content at R_5 consistently showed lower phytate content at both R_6 and R_7 stages. From the foregoing discussion of the results obtained in this study, it should be inferred that the three genotypes P.I. 416771, P.I. 416982, and Emperor have a greater potential as a genetic source to be incorporated into breeding program to produce progenies with lower phytate content and desirable agronomic traits.

References

Erdman, J.W. and K.E. Weingartner. 1981. Nutrition aspects of fiber in soybean products. J. Amer. Oil Chem. Sci. 58:511-514.

Fehr, W.R. C.E. Cavines, D.T. Burmmod, and J.S. Pennington. 1971. Stage of development description of soybean [Glycine max (L.) Merr.]. Crop Sci. 11:929-931.

Gupta, A. K. and A.D. Deodhar. 1975. Trypsin inhibitor activity in soybean [Glycine max (L.) Merr.]. Indian J. Nutri. 12:81-84.

Hafez, Y.S., A. Mohamed, P.A.J. Perera, G. Singh, and J.S. Hussein. 1989. Effect of Microwave heating and gamma irradiation on phytate and phospholipid contents of soybean [Glycine max (L.) Merr.]. J. Food Sci. 50(5):1271-1274.

Mohamed, A., Perera, P.A.J, and Y. Hafez. 1986. New chromophre for phytic acid determination. Cereal Chem. 63:475-476.

Mohamed, A. T. Mebrahtu, and M. Rangappa. 1991. Nutrient composition and anti-nutritional factors in selected vegetable soybean [Glycine max (L.) Merr.]. Plant Food For Human Nutri. 41:89-100.

Morse, W.J. 1950. Chemical composition of soybean seed in soybean products. I.K.S. Markely (ed.) John Wiley and Sons, N. Y.

Reddy, N.R., C.V. Blakrisham, and D.K. Salaunkhe. 1978. Phytate phosphorus changes during germination and soaking black bean (Phaseolus vulgaris L.) seed. J. Food Sci. 43:540-543.

Roboy, V., D.W. Dickinson, and F.E Below. 1984. Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of Glycine max

and G. soja. Crop Sci. 24:431-434.

Snyder, H.E. and T.W. Kwon. 1987. Soybean Utilization. Van Norstrand Reinhold. New York., N.Y.

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Test for Nuclear-Cytoplasmic Interaction

In a series of papers we have discussed conditional lethality involving soybean cytoplasmic mutants and nuclear mutants (Palmer and Cianzio, 1985; Palmer, 1992; Palmer and Minor, 1994). These results are summarized in Table 1.

Table 1. Phenotype of F_2 plants from crosses of cytoplasmic mutants as female parents and nuclear mutants as male parents. (Data from Palmer and Cianzio, 1985; Palmer, 1992; Palmer and Minor, 1994).

Female parent	Male parent		
	k_2k_2 (T239)	k_2k_2 $y_{20}y_{20}$ $Mdh_{1\text{-}n}$ $Mdh_{1\text{-}n}$ (T253)	y_{20} y_{20} $Mdh_{1\text{-}n}$ $Mdh_{1\text{-}n}$ (T323)
	Phenotype of homozygous recessive F_2 progeny		
cyt-Y_2 (T275)	viable	conditional lethal	conditional lethal
cyt-Y_3 (T278M)	viable	conditional lethal	conditional lethal
cyt-Y_4 (T314)	viable	conditional lethal	conditional lethal
cyt-Y_5 (T315)	viable	conditional lethal	conditional lethal
cyt-Y_6 (T316)	viable	conditional lethal	conditional lethal
cyt-Y_7 (T319)	viable	conditional lethal	conditional lethal
cyt-Y_8 (T320)	viable	conditional lethal	conditional lethal

Cytoplasmic yellow foliar mutants, cyt-Y_2 through cyt-Y_8, when used as female parent, give conditional lethals in the F_2 generation when crossed with k_2 y_{20} $Mdh_{1\text{-}n}$/k_2 y_{20} $Mdh_{1\text{-}n}$ or y_{20} $Mdh_{1\text{-}n}$/y_{20} $Mdh_{1\text{-}n}$ plants. Two independent nuclear mutational events, T253 and T323, give the same result. These genotypes are lethal under field conditions, but plants survive in reduced light under shade cloth in the glass house. The

reciprocal cross, nuclear mutants as female parent and cytoplasmic mutants as male parent give normal (expected) segregation ratios in the F_2.

Cytoplasmic yellow foliar mutants cyt-Y_2 through cyt-Y_8, when used as female parent and crossed with k_2/k_2, give all yellow foliar viable progeny in the F_2. Thus we do not know if the nuclear-cytoplasmic interaction with cyt-Y_2 to cyt-Y_8 is due to Mdh_{1-n}/Mdh_{1-n}, y_{20}/y_{20} or $y_{20}\ Mdh_{1-n}/y_{20}\ Mdh_{1-n}$.

In routine screening of newly acquired soybean germplasm from China, two accessions were identified that were green plants and Mdh_{1-n}. Our objective was to make reciprocal crosses of cyt-Y_2, cyt-Y_4, cyt-Y_6, and cyt-Y_8 with these two accessions (designated A93-125 and A93-309) to test for conditional lethality between Mdh_{1-n} and four cytoplasmic yellow folliar mutants.

Material and Methods

The genetic stocks used in these experiments are listed in Table 2. Reciprocal pollinations were obtained for all genetic combinations. Cross pollinations and progeny testing were done at the Bruner Farm, Ames, Iowa, and at the University of Puerto Rico - Iowa State University Soybean Nursery at Isabela, Puerto Rico.

Table 2. Soybean genetic stocks used to test for nuclear-cytoplasmic interaction

Genetic type or identity number	Gene symbol	Phenotype
T275	cyt-Y_2	Yellowish leaves, becoming greenish yellow
T314	cyt-Y_4	Yellowish leaves
T316	cyt-Y_6	Yellowish leaves, vigorous
T320	cyt-Y_8	Greenish yellow leaves
A93-125	Mdh_{1-n}	Green leaves, Mdh_1 absent
A93-309	Mdh_{1-n}	Green leaves, Mdh_1 absent

Seed of the parents and the F_2 and F_3 generations were analyzed for MDH by using starch gel electrophoresis (Cardy and Beversdorf, 1984; Weeden and Wendel,

1989). The seedlings were transplanted to peat pots and allowed to harden off for several days before transplanting to the field. Goodness of fit between observed and expected F_2 ratios for MDH present : MDH absent was determined by using chi-square tests.

Results

In the control populations the data were compatible with the expected results (Table 3). In all crosses the ratio of MDH present : MDH absent was 3:1. All $\underline{Mdh_{1-n}}$ plants were viable and had green foliage.

Table 3. Phenotype and number of F_2 plants from crosses of nuclear mutants as female parents and cytoplasmic mutants as male parents: control populations.

Female parent	Male parent	MDH present	MDH absent	χ^2 (a) (3:1)
		Number of F_2 Plants		
A93-125	$\underline{cyt\text{-}Y_2}$	73	27	0.21
A93-125	$\underline{cyt\text{-}Y_4}$	108	32	0.34
A93-125	$\underline{cyt\text{-}Y_6}$	89	31	0.04
A93-125	$\underline{cyt\text{-}Y_8}$	73	25	0.01
A93-309	$\underline{cyt\text{-}Y_2}$	77	23	0.21
A93-309	$\underline{cyt\text{-}Y_4}$	75	24	0.03
A93-309	$\underline{cyt\text{-}Y_6}$	73	27	0.21
A93-309	$\underline{cyt\text{-}Y_8}$	108	35	0.02

(a) χ^2 value of 3.84 is significant at the 0.05 probability level, 1 df.

In the experimental populations the data were compatible with the expected results (Table 4). In all the crosses the ratio of MDH present : MDH absent was 3:1. All $\underline{Mdh_{1-n}}$ plants were viable and had yellow foliage.

Table 4. Phenotype and number of F_2 plants from crosses of cytoplasmic mutants as female parents and nuclear mutants as male parents: experimental populations.

Female parent[a]	Male parent	MDH present[a]	MDH absent[a]	$\chi^{2\,b}$ (3:1)
		Number of F_2 plants		
cyt-Y_2	A93-125	221	78	0.19
cyt-Y_4	A93-125	131	38	0.57
cyt-Y_6	A93-125	229	71	0.28
cyt-Y_8	A93-125	221	79	0.28
cyt-Y_2	A93-309	259	80	0.35
cyt-Y_4	A93-309	205	74	0.35
cyt-Y_6	A93-309	215	65	0.48
cyt-Y_8	A93-309	227	73	0.07

[a] All plants had yellow leaves.

[b] χ^2 value of 3.84 is significant at the 0.05 probability level, 1 df.

Discussion

We have shown that the two phenotypes, yellow foliage (Y_{20} locus) and malate dehydrogenase (MDH locus), cosegregate and are inherited as single gene recessives (Hedges and Palmer, 1992). The natural occurrence of green foliar plants that are $Mdh_{1\text{-}n}$ (A93-125 and A93-309) allowed us to test for nuclear - cytoplasmic conditional lethality independently of y_{20}.

On the basis of our results, we conclude that a nuclear-cytoplasmic interaction does not occur between cyt-Y_2, cyt-Y_4, cyt-Y_6, and cyt-Y_8, in combination with A93-125 and A93-309 as male parents. These two male parents have green foliage (Y_20) and are $Mdh_{1\text{-}n}$. Thus the genotype cyt-Y_2 $Mdh_{1\text{-}n}/Mdh_{1\text{-}n}$ is viable but cyt-Y_2 y_{20} $Mdh_{1\text{-}n}/y_{20}$ $Mdh_{1\text{-}n}$ is conditional lethal (Palmer, 1992). Genetic tests with cyt-Y_3, cyt-Y_4, cyt-Y_5, cyt-Y_6, cyt-Y_7, and cyt-Y_8 and y_{20} $Mdh_{1\text{-}n}/y_{20}$ $Mdh_{1\text{-}n}$ plants also indicated conditional lethality (Palmer and Minor, 1994).

Interactions leading to conditional lethality occur only in the presence of the cyt-Y

mutations along with the y_{20} $Mdh_{1\text{-}n}$ mutations. Crosses of the cyt-Y mutations with the $Mdh_{1\text{-}n}$ mutations give all viable F_2 plants. Crosses of the cyt-Y mutations with the y_{20} mutations are necessary to determine the cause of the interaction. Genetic recombination tests are underway to obtain the y_{20} y_{20} Mdh1 Mdh1 (yellow foliage, MDH present) genotype.

References

Cardy B.J., and W.D. Beversdorf. 1984. A procedure for the starch gel electrophoretic detection of isozymes in soybean [Glycine max (L.) Merr.]. Dep. Crop Sci. Tech. Bull. 119/8401. University of Guelph, Ontario, Canada.

Hedges, B.R. and R.G. Palmer. 1992. Inheritance of malate dehydrogenase nulls in soybean. Biochemical Genetics 30:491-502.

Palmer, R.G. and S.R. Cianzio. 1985. Conditional lethality involving nuclear and cytoplasmic chlorophyll mutants in soybeans. Theoretical and Applied Genetics 70:349-354.

Palmer, R.G. 1992. Conditional lethality involving a cytoplasmic mutant and chlorophyll-deficient malate dehydrogenase mutants in soybean. Theoretical and Applied Genetics 85:389-393.

Palmer, R.G. and V.C. M. Minor. 1994. Nuclear-cytoplasmic interaction in soybean, Glycine max (L.) Merr. American Journal of Botany 81:997-1003.

Weeden, N.D., and J.F. Wendel. 1989. Genetics of plant isozymes. In D.F. Soltis and P.S. Soltis (eds.), Isozymes in Plant Biology, pp 46-72. Dioscorides Press, Portland, OR.

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Putative associations between loci controlling palmitic acid content and stem termination type in soybean

Consumer and food processing groups have expressed concern over the nutritional quality of soybean oil (Wilson, 1991). This attention has directed interest towards the development of oils with reduced saturated fatty acid content. Medical studies have shown that diets hinge in saturated fatty acids may increase blood serum cholesterol levels, and heighten the risk of coronary heart disease (Willett, 1994). Palmitic acid is the predominant saturated fatty acid in soybean and most other vegetable oils (Weiss, 1983). A single major allele was identified by USDA soybean breeders in North Carolina to reduce palmitic acid content in soybean oil (Rebetzke, 1994; Wilcox et al., 1994). We observed in field studies that lines homozygous for this low palmitic acid allele are often determinate in growth habit. The objective of this study was to determine if genes controlling reduced palmitic acid content are associated with stem termination type in populations segregating for both characteristics.

Materials and Methods

Determinate low palmitic acid germplasm, N87-2122-4 (53 g kg^{-1} oil palmitic acid), was crossed to two indeterminate normal palmitic acid soybean cultivars, Kenwood and P9273 (123 and 108 g kg^{-1} palmitic acid, respectively). F_1 plants were self-pollinated in Puerto Rico and F_2 populations grown at Clayton, North Carolina in 1991. Lines were inbred through single-seed descent to the F_5 generation, and then bulk-selfed a further two generations. Maturity group IV lines were sampled at random from each cross and grown in three replications at Clayton, North Carolina during 1993 for determination of oil composition and growth habit. Stem termination type was recorded at maturity as determinate, semi-determinate, or indeterminate following Bernard (1972). Seeds were sampled from each line and palmitic acid content determined following Rebetzke (1994).

The discrete and ordered nature of data collected on stem termination type resulted in a nonnormal distribution of errors hence making them unsuitable for analysis of variance (Steel and Torrie, 1980). Accordingly, these data were analyzed following nonparametric statistical procedures (Sprent, 1993). Numeric scored 0, 1, and 2 were assigned to determinate, semi-determinate, and indeterminate genotypes. Kendallís coefficient of rank correlation (r_B) was estimated using the tauB option of SAS procedure CORR (SAS, 1988) to relate variation in palmitic acid content with stem type for lines grown at Clayton, NC. The semi-determinate class was then removed, and a studentís t-test conducted for differences in palmitic acid content between determinate and indeterminate growth habits.

Results and Discussion

Large variation was observed among lines for palmitic acid content and stem termination type (Table 1). Kendallís r_B for stem type and palmitic acid content ranged between 0.29 ($P < 0.01$, n=44) and 0.04 ($P > 0.05$, n=43) for the Kenwood- and P9273-derived populations, respectively. Variances of determinate and indeterminate populations were approximately equal based on F_{max} tests (Sokal and Rohlf, 1969) for lines derived from Kenwood and P9273. A comparison was made of palmitic acid means for the determinate and indeterminate growth habit classes. For the Kenwood populations mean palmitic acid content or the determinate lines (79 g kg^{-1}) was significantly smaller ($P < 0.01$) than the mean for the indeterminate lines (101 g kg^{-1}). However, means were not significantly different ($P > 0.05$) for lines derived from P9273 (cf. 81 and 82 g kg^{-1} for low and normal palmitic acid content, respectively). Associations appear to be population dependent. Furthermore, where significant associations exist, they appear to be small and suggest a loose genetic linkage among loci conditioning the two characteristics. Evidence for such linkage supports earlier observations of a significant ($P < 0.01$), positive genetic correlation between palmitic acid content and plant height among sets of near-isogenic palmitic acid soybean bulks (Rebetzke unpublished data). Further investigation is needed to verify if the poor association among the P9273-derived lines is real, or a consequence of sampling reduced numbers of indeterminate lines for this cross.

Breeders wishing to incorporate low palmitic acid alleles into indeterminate backgrounds may need to increase the sizes of early segregating populations so as to recover lines combining low palmitic acid content with desired growth habits.

Frequencies of lines containing desired growth habit alleles may be increased following a minimum single backcross to the adapted parent. We recommend at least one backcross as this would also enable the breeder to increase the frequency of alleles conditioning high seed yield prior to inbreeding and selection.

Table 1. Means standard errors (S.E.), and ranges of palmitic acid content or determinate, semi-determinate, and indeterminate lines from two separate soybean crosses.

Cross	N87-2122-4 x Kenwood			N87-2122-4 x)9273		
	D+	SD	I	D	SD	I
	Palmitic acid content (g kg^{-1} oil)					
Mean ± S.E.	79 ± 5.8	85 ± 6.7	101 ± 5.9	81 ± 4.4	81 ± 4.4	82 ± 8.8
Range	58 - 116	58 - 115	64 - 124	51 - 107	55 - 104	60 - 106
Number of lines	17	15	12	22	15	6

+D, SD, and I refers to determinate, semideterminate, and indeterminate growth habit classes, respectively.

References

Bernard, R.L. 1972. Two genes affecting stem termination type in soybeans. Crop Sci. 12:235-9.

Rebetzke, G.J. 1994. Inheritance and stability of palmitic acid content in soybean. (Ph.D. dissertation, North Carolina State University, Raleigh, NC).

SAS, 1988. SAS procedures guide for personal computers. Release 6.03 1st ed. SAS Institute Inc., Cary NC.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. The principles and practice of statistics in biological research. 1st ed. W.H. Freeman and Co., San Francisco.

Sprent, P. 1993. Applied nonparametric statistical methods. 2nd ed. Chapman and Hall, London.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach, 2nd ed. McGraw-Hill Book Co., New York.

Weiss, T.J. 1983. Food oils and their uses. 1st ed. AVI Publishing Co. Inc., Westport, Connecticut.

Wilcox, J.R., J.W. Burton, G.J. Rebetzke, and R.F. Wilson. 1994. Transgressive segregation for palmitic acid in seed oil of soybean. Crop Sci. 34:1248-50.

Willett, W.C. 1994. Diet and Health: What should we eat? Science 264:532-7.

Wilson, R.F. 1991. Advances in the genetic alteration of soybean oil composition. p. 38-52. In R.F. Wilson (ed.) Designing value-added soybeans for markets of the future. Amer. Oil Chem. Soc., Champaign, IL.

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RAPD analysis using backcross-derived line in mapping genes for resistance to Heterodera glycines 1. Race 3 in Peking cultivar

Near-isogenic lines (NILS) are an important source of germplasm in mapping experiments and linkage analysis. The NEL gene mapping technique is based on the assumption that when a conventional marker is introgressed from a non-recurrent parent or donor parent (DP) into a recurrent parent (RP) to create a NEL via the backcross breeding method (Fehr, 1987), a small number of DP-derived markers will be located on the introgressed chromosome, with the remaining markers randomly located on the other non-marker chromosomes (Muehibauer et al., 1989). Traditionally, the backcross method has been extensively used for developing disease and pest resistant cultivetrs.

With the development of molecular techniques, NELs have been prevalently employed in the identification of molecular markers associated with disease or pest resistance. Williamson et al., (1994) reported a PCR-based marker linked to the nematode resistance gene, Mi, in tomato using several pairs of NILs differing in nematode resistance and the extent of introgressed DNA from Lycopersicon igeruvianum resistant to Meloidogyne incognita. Barua et al., (1993) identified a RAPD marker linked to Rh locus conferring resistance to Rhyncosporium secalis in barley screening a pair of NILs using 300 decamer primers. Michelmore et al., (1991) identified 10 RAPD and 4 RFLP markers linked to genes for resistance to downy mildew (Dm) in lettuce using NILS. Young et al., (1988) used RP/NIIL/DP sets and identified 2 RFLP loci that were tightly linked to Tm-2a locuswhich confers resistance to tobacco mosaic virus in tomato. Osborn et al., (1987) found a RFLP locus linked to the QTL controlling soluble solids content in tomato fruit using BC_5S_5 NIL.

The objective of this study was to identify PCR-generated amplified polymorphic DNA transmitted frcim Peking to NC-55. RAPD marker analysis was conducted with Lee (RP)-NC55 (NIL) pair. Sutisequently identified polymorphisms were used for segregation analysis in the Peking x Essex F_2 population to expedite the mapping of loci resistant to the soybean cyst nematode Heterodera glycines race 3.

Materials and methods

Lee (RP) was backcrossed to Peking (DP) 3 times to derive NC55 (Bernard et al., 1988). The genes for resistance transferred from Peking to NC5 5 are Rhg_4 (linked to i) and probably rhg_1, rhg_2 and rhg_3 (Bernard et al., 1988). Essex (derivative of RP) and Peking (DP) were used to derive the mapping population.

Total genoniic DNA was extracted using the CTAB method (Rogers and Bendich, 1985) with modifications fbr soybeans as outlined by Keim et al., 1988. RAPD analysis was conducted as described before (Skorupska et al., 1994). Three hundred and ninety-seven primers (Operon Technologies Inc., Alameda, CA) were used for screening the RP/NIL. The polymorphic primers were used to screen the parental lines and segregating population.

Results and Discussion

NC55 derives its resistance to SCN from Peking, the resistant donor parent (Bernard et al., 1988). The highly significant association between the region around i locus and resistance to SCN race 3 in Peking has been reported elsewhere (Mahalingam and Skorupska, 1995, in press). Essex, the susceptible parent of the mapping population, has Lee (RL) in its pedigree (Allen and Bharadwaj, 1984). The assumption of the comparison experiment was that the molecular polymorphism that matched the NC55-Peking pattem and shows the opposite pattem in Lee-Essex pair can be useful in identifying linkages with SCN resistance.

Three hundred and ninety-seven decamer primers were employed for screening Lee and NC55 (Table 1). Nine primers out of 397 did not give any products under our amplification conditions. A total of 3034 readable bands were generated at the average rate of 8 bands/primer. About 50% primers produced polymorphisms between Lee and Peking.

In this research, we were interested in the polymorphism level between donor, recurrent parent, and NC55. Sixty-one primers generated polymorphisms between NC55 and Lee and they produced a total of 72 polymorphic bands, 2.4 % of total number of bands (Table 1). Forty-four of these polymorphic primers had the DP pattern in NC55. In the next step, Peking and Essex, parents of the F_2 mapping population, were screened vith these primers. Seventeen polymorphisms matched the required comparison pattem and showed an identical banding pattem for NC55-

Peking and the opposite pattern for Lee-Essex (Table 2). These polymorphisms were tested in the F_2 populations of Peking and'PI 437654 crosses with Essex. One polymorphism, S07a (1.57kb) showed a highly significant association with the cyst nematode response and was found to be linked to the i locus (Mahalingam and Skorupska, 1995, in press). The polymorphisms generated by B15, C08, EO 1, and II 3 primers mapped to the same linkage group of the Clemson University RAPD linkage map (Choi and Skorupska, 1995, in submission). Molecular analysis of genetically close related germplasms in soybean can be useful for mapping of targeted genomic regions.

Table 1. RAPD polymorphisms between NC55 and Lee.

Number of primers tested: 397
Total number of bands generated: 3034
Number of polymorphic primers: 61
Number of polymorphic bands: 72 (2.4% of the total number of bands)
Number of polymorphic bands between NC55 and Lee that correspond with Peking and Essex pattems: 17 (0.56% of the total number of bands and 23.6% of the polymorphic bands)

Table 2. Polymorphic RAPD bands between Lee and NC55 that correspond to the opposite pattems in Essex and Peking.

	PRIMER	LEE	NC55	PEKING	ESSEX	M.W. (in kb)
I	AII	+	-	-	+	1.52
2	BOI	+	-	-	+	1.55
3	B15	+	-	-	+	0.58
4	C02	-	+	+	-	0.69
5	C08	-	+	+	-	0.85
6	C15	-	+	+	-	0.98
7	D06	-	+	+	-	0.83
8	E01a	+	-	-	+	1.90

Table 2 continued

	PRIMER	LEE	NC55	PEKING	ESSEX	M.W. (in kb)
9	E01b	-	+	+	-	1.80
10	101	-	+	+	-	1.15
11	110	-	+	+	-	1.10
12	I13	+	-	-	+	0.79
13	I14	+	-	-	+	0.45
14	K06	+	-	-	+	1.32
15	M18	-	+	+	-	1.30
16	S07a	+	-	-	+	1.57
17	S07b	-	+	+	-	1.55

References

Allen, E.L. and H.L. Bharadwaj. 1987. Genetic relationships and selected pedigree diagrams of North American soybean cultivars. The University of Tennessee, Agricultural Experiment Station, Knoxville, TN. Bulletin No 652, p 1-3.

Barua, U.M., K.J. Chalmers, C.A. Hackett, W.T.B. Thomas, W. Powell, and R. Waugh. 1993. Identification of RAPD markers linked to a Rhyncosporium secalis resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity 71:177-184.

Bernard, R.L., G.A. Juvik, E.E. Hartwig, and C.J. Edwards, Jr. 1988. Origins and pedigrees of public soybean varieties in the United States and Canada. U. S. Department of Agriculture, Technical Bulletin No. 1746, p. 61.

Choi, I.S. and H.T. Skorupska. 1995. Random amplified polymorphic DNA map for intraspecific cross of Essex x PI 437654 in soybean, Glycine max (L.) Merr. (In submission).

Fehr, W.R. 1987. Principles of cultivar development. Vol 1. Theory and technique. MacNEllan Publ. Co., NY.

Mahalingam, R. and H.T. Skorupska. 1994. DNA markers for resistance to Heterodera glycines 1. race 3 in soybean cultivar Peking. Breeding Science. (In press).

Muehlbauer, G.J., J.E. Specht, M.A. Thomas-Compton, P.E. Staswick, and R.L. Bernard. 1988. Near isogenic lines a potential resource in the integration of conventional and molecular marker linkage maps. Crop Sci. 28:729-735.

Muehibauer, G.J., J.E. Specht, P.E. Staswick, G.L. Graef, and M.A. Compton. 1989. Application of the near-isogenic line mapping technique to isozyme markers. Crop Sci. 29:1548-1553.

Osbom, T.C., D.C. Alexander, and J.F. Fobes. 1987. Identification of restriction fragment length polymorphism linked to genes controlling soluble solids content in tomato fruit. Theor. Appl. Genet. 73:350-356.

Paran, I., R. Kesseli, and R. Kfichelmore. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34:1021-1027.

Rogers, S.0 and A.G. Bendich. 1985. Extraction of DNA from milligrain amount of fresh, herbarium and munimified plant tissue. Plant Mol. Biol. 5:69-76.

Skorupska, H.T., I.S. Choi, A.P. Rao-Arelli, and W.C. Bridges. 1994. Resistance to SCN and molecular polymorphism in various sources of Peking soybean. Euphytica. 75:63 -70.

Williamson, V.M., J.Y. Ho, F.F. Wu, N. Niller, and I. Kaloshian. 1994. A PCR-based marker tightly linked to the nematode resistance gene, Mi, in tomato. Theor. Appl. Genet. 87:757763.

Young, N.D., D. Zamir, M.W. Ganal, and S.D. Tanksley. 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked the Tm-2a gene in tomato. Genetics 120:579-585.

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Bulk segregant analysis for identification of RAPD markers associated with resistance to Heterodera glycines I. on linkage group A in Peking cultivar

Bulk segregant analysis (BSA) is a PCR based strategy to identify molecular markers utilizing two bulked DNA samples gathered from individuals in a single segregating population (Michelmore et al., 1991). Each bulk is composed of individuals that differ for a specific phenotype or genotype. The rationale behind BSA being that for simple genetic traits, all loci in the genome should appear to be in linkage equilibrium except in the region of the genome linked to the selected locus. Markers linked to this locus should appear polymorphic between the pools for parental alleles. This technique was employed successfully to target markers to the Dm 518 locus in lettuce which governs resistance to downy mildew disease (Michelmore et al., 1991). A pooling strategy based on known RFLP genotypes from existing mapping populations was used to target chromosomal intervals. It was used successfully to target RAPD markers to the region of the tomato genome responsible for fruit ripening and pedicel abscission (Giovannoni and Wing, 1991). A similar pooling strategy was employed to identify 100 RAPD markers specific to chromosome 1, of Arabidopsis thaliana (Reiter et al., 1992). In an experiment with near isogenic lines of barley, 300 decamers were screened to establish associations between molecular markers and resistance to Rhyncosporium secalis. One primer was identified which generated a product from the donor parent and the Rhyncosporium-resistant NIL, but not from the recurrent parent. The presence or absence of this product along with the resistance phenotype was used to create two pools of DNA and these were screened with 700 primers. Seven products were identified which were present in the resistant bulk and parent but were absent in the susceptible samples. Segregation analysis established their association with resistance to Rhyncosporium, conditioned by Rh locus localized on the long arm of chromosome 3 (Barua et al., 1993).

We detected major QTL for SCN resistance localized in the proximity of the i locus, self dark seed coat phenotype, in the Peking and Essex cross. Importance of i locus in relation to SCN resistance has been reported (Mahalingam and Skorupska,

1995, in press). In this experiment, bulk analysis was employed to facilitate identification of RAPD markers linked to the i locus, and enhance genetic mapping in the region containing the major QTL, associated with resistance to SCN in Peking.

Materials and Methods

The F_2 mapping population was derived by selfing the F_1, from a cross between Peking and Essex. The F_2 genotypes for the seed coat color trait were confirmed by analyzing the $F_{3:4}$ and in a few cases $F_{4:5}$, lines were observed for segregation at i locus. Genotypes were classified as ii, i^ji, or i^ji^j.

Total genomic DNA was extracted using the CTAB method (Rogers and Bendich, 1985) with modifications for soybeans as outlined by Keim et al., 1988. Twelve homozygous black seeded ii individuals and 10 homozygous yellow i^ji^j individuals were selected. Equal volumes of DNA of the same concentration (60 ng/µl) from each individual were pooled to obtain two bulks. Four hundred and two decamer primers (Operon Technologies, Inc., Alameda, CA) were used to screen the two bulks. RAPD analysis was conducted as described previously (Skorupska et al., 1994).

Results and Discussion

Quantitative trait loci (QTL) tagging by pooling strategies utilizing the PCR technology may not be effective unless the QTL's have a very large effect (Wang and Paterson, 1994). Given this caveat and the fact that the heritability of the SCN female indices depend on the homogeneity of tested segregants (unpublished results), we refrained from bulking the DNA based on a qualitative approach. A dominant gene for resistance to SCN (Rhg_4) in Peking has been reported to be tightly linked to the i locus (0.35 cM, Matson and Williams, 1964). In our research, we estimated a dominant QTL for SCN resistance in Peking to be localized 1.2 cM from the i locus using the MAPN=R/QTL program (Mahalingam and Skorupska, in submission).

One hundred and sixteen primers were polymorphic of the 392 primers screened between two contrasting bulks for i locus (Table 1). A total of 3268 bands were generated at the rate of 8 bands/primer. Ten primers did not produce any products under our amplification conditions. One hundred and sixty=six bands (5.07%) of the total number of bands generated among the bulks were polymorphic.

Table 1. RAPD polymorphisms in the i locus bulk analysis.

Number of primers tested: 402
Total number of bands generated: 3268
Number of polymorphic primers: 116
Number of polymorphic bands: 166 - 5.07% of total number of bands
Number of polymorphic bands between Peking and Essex that match the ii and i^1i^1 bulks patterns:
24 - 0.73% of the total number of bands
12.05% of the polymorphic bands

In a subsequent step, polymorphic bands contrasting for i locus bulks were compared with the patterns generated in parental cultivars, Peking and Essex. Twenty-four polymorphic bands fit the Peking-black bulk pattem vs. Essex-yellow bulk pattern (Table 2). Among these 24 bands, three polymorphisms generated by BOI, S07 and W03 primers confirm previously selected polymorphisms from screening the Peking germplasms (Skorupska et al., 1994). Primers C15, EO1, I13, K06, and S07 generated bands that fit the polymorphisms transmitted from Peking to NC55 with Lee as a recurrent parent (Mahalingam and Skorupska, 1995).

The 24 polymorphic DNA bands were evaluated in the F_2 mapping population for their association with SCN resistance. The S07 polymorphism was found to be significantly associated with the nematode response and was localized 17 cM from the i locus (Mahalingam and Skorupska, 1995, in press). At a significance level of 95%, polymorphisms generated by K07, T12, and W03 were also linked to the i locus (39 cM, 37 cM, and 36 cM, respectively). The polymorphisms generated by RAPD bulk analysis for Peking and Essex cross was 12.5% (Table 2). The polymorphism generated by S07 primer was detected in a previous survey of various Peking germplasms (Skorupska et al., 1994), an isoline survey (Mahalingam and Skorupska, 1995), in Peking x Essex F_2 population (Mahalingam and Skorupska, 1995, in press), in Essex x PI 437654 F_2 population (Choi and Skorupska, in submission) and bulks of race 3 resistant and race 3 susceptible cultivars. Thus, the pooling strategy proved to be a useful technique to identify markers linked to a target gene. The S07 primer can be used as a diagnostic marker for QTL SCN resistance on the linkage group A of the ISU/USDA molecular soybean map.

Table 2: Polymorphic RAPD loci between $\underline{ii}$ and $\underline{i^{i}i^{i}}$ bulks that correspond to Peking and Essex patterns.

	PRIMER	$\underline{ii}$	$\underline{i^{i}i^{i}}$	PEKING	ESSEX	MW (in kb)
1.	All	-	+	-	+	1.52
2.	B03	+	-	+	-	1.20
3.	B12	-	+	-	+	0.90
4.	C08	+	-	+	-	0.85
5.	C15	+	-	+	-	0.98
6.	D06	+	-	+	-	0.83
7.	E01a	-	+	-	+	1.90
8.	F16	+	-	+	-	0.55
9.	G04	+	-	+	-	0.14
10.	G08	+	-	+	-	0.40
11.	113	+	-	+	-	0.79
12.	K03	+	-	+	-	0.45
13.	K06a	-	+	-	+	1.32
14.	K07b	+	-	+	-	0.51
15.	L12	-	+	-	+	0.55
16.	M13	-	+	-	+	0.56
17.	S07a	-	+	-	+	1.57
18.	S07b	+	-	+	-	1.55
19.	T12a	-	+	-	+	0.72
20.	T14	+	-	+	-	0.88
21.	T17	-	+	-	+	0.83
22.	AH9a	-	+	-	+	0.42
23.	AH9b	+	-	+	-	0.40
24.	AW14	-	+	-	+	0.45

References

Barua, U.M., K.J. Chalmers, C.A. Hackett, W.T.B. Thomas, W. Powell, and R. Waugh. 1993. Identification of RAPD markers linked to a Rhyncosporium secalis resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity 71:177-184.

Choi, I.S. and H.T. Skorupska. 1995. Detection of molecular markers for resistance to Heterodera glycines I., races 3, 5, 14 in Essex X PI 437654 cross. Crop Science. (In submission.)

Giovannoni, J.J. and R.A. Wing. 1991. Isolation of molecular markers for specific chromosomal intervals using DNA pools for existing population. Nucleic Acids Res. 19:6553-6558.

Mahalingam, R. and H.T. Skorupska. 1994. DNA markers for resistance to Heterodera glycines 1. race 3 in soybean cultivar Peking. Breeding Science. (In press.)

Matson, A.L. and L.F. Williams. 1965. Evidence of a fourth gene for resistance to the soybean cyst-nematode. Crop Sci. 5:477.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific gnomic regions using segregating populations. PNAS. 88:9828-9832.

Reiter, R.S., J.G.K. Williams, K.A. Feldman, J.A. Rafalski, S.V. Tingey, and P.A. Scolnik. 1992. Global and local genome mapping in Arabidopsis thaliana by using RIL and RAPD. Proc. Natl. Acad. Sci. 89:1477-1481.

Rogers, S.O. and A.G. Bendich. 1985. Extraction of DNA from milligram amount of fresh, herbarium and mummified plant tissue. Plant Mol. Biol. 5:69-76.

Skorupska, H.T., I.S. Choi, A.P. Rao-Arelli, and W.C. Bridges. 1994. Resistance to SCN and molecular polymorphism in various sources of Peking soybean. Euphytica. 75:63-70.

Wang, G.L. and A.H. Paterson. 1994. Assessment of DNA pooling strategies for mapping of QTLs. Theor. Appl. Genet. 88:355-361.

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Identification of PCR-generated DNA variation in transmission analysis with P.I. 437654

Soybean line P.I. 437654 was reported to be resistant to all races and biotypes of soybean cyst nematode (SCN), Heterodera glycines I., collected in the USA (Anand et al., 1988; Anand, 1991). 'Hartwig' was developed from the cross of Forrest[3] X P.I. 437654 as the donor of genes for resistance to SCN, and initially released as S88-2036, a soybean line with multiple-race resistance to soybean cyst nematode (Anand, 1991, 1992).

We have constructed the RAPD map for cross of Essex x P.I. 437654. This map consists of 27 linkage groups with more than 160 molecular markers assigned. Eight RAPD linkage groups were integrated with RFLP molecular public map (Choi and Skorupska, 1995). The strategy employed for molecular marker selection for mapping procedure was based on: (i) polymorphisms of RFLP markers between Essex and P.I. 437654 and (ii) polymorphisms of RAPD markers between Hartwig and 'Forrest'; P.I. 88788, 'Peking', and P.I. 437654, and polymorphisms between contrasting bulks of resistant and susceptible cultivars for races 3, 5, and 14. Here, we report the polymorphisms between Hartwig and Forrest.

Two hundred eighty-eight decamer primers (Operon Technologies, Inc., Alameda, CA) were used. RAPD analyses were conducted as described previously (Skorupska et al., 1994). Twenty-seven primers generated well resolved polymorphisms between Hartwig and Forrest (Table 1). These polymorphisms were compared with RAPD patterns in P.I. 437654. DNA bands which showed identical patterns between Hartwig and P.I. 437654 but different from Forrest (Peking was donor of genes for resistance to SCN race 3 and 5 in Forrest) were screened for polymorphisms with Essex (Table 2). The identified DNA banding patterns reflect genomic variation transmitted from P.I. 437654 to cv. Hartwig, and might be associated with traits of agronomic importance, e.g. soybean cyst nematode resistance and/or sudden death syndrome.

Table 1. RAPD polymorphisms between Hartwig and Forrest.

Numbers of primers tested:	288
Total number of bands:	2160
Bands per primer:	7.5
Number of polymorphic primers:	27
Percentage of polymorphic primers:	9.4
Number of polymorphic bands:	33
Percentage of total number of bands:	1.53
Percentage of bands of polymorphic primers:	15.6

Table 2. RADP polymorphism transmitted from P.I. 437654 to Hartwig.

Primer	Molecular weight (bp)	Hartwig	Forrest	PI 437654	Essex
A20	1580	0	1	0	1
B05	1350	0	1	0	1
C08a	650	1	0	1	0
C08b	610	0	1	0	1
C09	1100	0	1	0	1
C12	1150	0	1	0	0
C20	740	1	0	1	1
C20b	700	0	1	0	1
D20	1660	0	1	0	0
E03	1580	0	1	0	0
F02	2080	0	1	0	0
F07	980	1	0	1	1
G05	1380	0	1	0	1
H04a	890	1	0	1	0
H04b	830	0	1	0	0
H05	980	1	0	1	0
H07	1330	1	0	1	0
H13	1430	1	0	1	1
K04	1220	1	0	1	0
K10	2200	0	1	0	0

Table 2. continued

Primer	Molecular weight (bp)	Hartwig	Forrest	PI 437654	Essex
L15	680	1	0	1	1
M20	880	1	0	1	0
N06	1470	0	1	0	1
N10	2100	0	1	0	1
N11a	1100	1	0	1	0
N11b	1000	0	1	0	1
N18	1320	1	0	1	1
S20	1470	1	0	1	0
W09	830	1	0	1	1
W12a	1870	0	1	0	1
W12b	1750	1	0	1	1
X16a	1000	1	0	1	1
X16b	900	0	1	0	0

1 - band present: 0 - band absent

References

Anand, S. C. 1992. Registration of 'Hartwig' soybean. Crop Science 32:1069-1070.

Anand, S.C. 1991 Registration of soybean germplasm line S88-2036 having multiple-race soybean cyst nematode resistance. Crop Science 31:856.

Anand, S.C., K.M. Gallo, I.A. Baker, and E.E. Hartwig. 1988 Soybean plant introductions with resistance to Races 4 or 5 of soybean cyst nematode. Crop Science 28:563-564.

Choi, I.S. and H. T. Skorupska. 1995. Random amplified polymorphic DNA map for intraspecific cross of Essex x P.I. 437654 in Glycine max. (In submission.)

Skorupska, H.T., I.S. Choi, A.P. Rao-Arelli, and W.C. Bridges. 1994. Resistance to SCN and molecular polymorphism in various sources of Peking soybean. Euphytica 75:63-70.

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RAPD marker selection using DNA bulk of cultivars for differential response to soybean cyst nematode races 3, 5, and 14

DNA pooling system has been used as a strategy for identifying DNA markers closely linked to gene(s) or genomic regions of interest. Michelmore et al., (1991) used the pooling strategy, bulk segregant analysis (BSA) of individuals of a common phenotype, and identified 3 RAPD markers linked to a gene for resistance to downy mildew disease in lettuce. Based on known RFLP genotypes from existing mapping populations for opposite parental alleles in a targeted chromosomal interval, Giovannoni et al., (1991) detected 2 RAPD markers tightly linked to loci affecting fruit ripening and pedicle abscission in tomato. Reiter et al., (1992) used the pooling strategy to identify 100 RAPD markers specific to chromosome 1 of Arabidopsis thaliana. Wang and Paterson (1994) assessed the DNA pooling strategy for mapping quantitative trait loci (QTL) and suggested that the use of phenotype-based DNA pools might be successful in tagging QTLs of very large effect, but is unlikely to permit comprehensive identification of the majority of QTLs affecting a complex trait.

In this paper, we present a modified approach of molecular marker identification to facilitate the mapping of complex traits. RAPD banding pattems from bulks of cultivars in response to soybean cyst nematode (SCN) races 3, 5, and 14; one bulk consisting of the DNA from resistant cultivars and another bulk of susceptible cultivars were compared with RAPD patterns of sources for resistance. This assay is different from the previous DNA pooling strategies in two ways: (i) each bulk for a specific phenotype was composed of DNAs of cultivars of various genetic backgrounds but sharing a common feature such as resistance or susceptibility to a race of SCN (bulk segregant analysis employs bulks of individuals that differ for a specific phenotype or genotype in a single segregating population), and (ii) this assay was used for identification of RAPD polymorphisms for their subsequent applications in different segregating populations to target a specific region of the genome.

Materials and Methods

Total genomic DNA was extracted using the CTAB method (Rogers and Bendich, 1985) with modifications for soybeans as outlined by Keim et al., (1988). Equal amounts of DNA of each cultivar (60 ng) was used to create the bulk of DNA (Table 1). RAPD analysis was conducted as described by Skorupska et al., (1994).

Table 1. Germplasms used for DNA bulks of resistant cultivars and susceptible cultivars to SCN races 3, 5, and 14.

Race 3	resistant	Bedford, Bradley, Centennial, Coker 368, Coker 6738, Coker 6847, Cordell, Epps, Forrest, Hartwig, Leflore, Lloyd, Kirby, P.I. 90763, Stonewall, Thomas, Twiggs
	susceptible	A6785, A7986, Bragg, Braxton, CNS, Hutcheson, Jackson, Kershaw, Lamar, Lee, Perrin, TracyM, Wright, Young
Race 5	resistant	Cordell, Hartwig, P.I. 90763
	susceptible	Bedford, Coker 6847, Hutcheson, Leflore, Lloyd, Stonewall, Thomas
Race 14	resistant	Bedford, Bradley, Epps, Hartwig, Leflore, Lloyd
	susceptible	Coker 368, Coker 6738, Cordell, Forrest, P.I. 90763, Stonewall, Thomas,

One hundred ninety-two decamer primers (Operon Technologies, Inc., Alameda, CA) were used for RAPD analysis of the contrasting DNA bulks of cultivars for SCN races 3, 5, and 14. Simultaneously, the same primers were used for screening of the susceptible cultivar, 'Essex', and resistant genotypes: P.I. 88788, 'Peking', and P.I. 437654. The detected polymorphisms from these two experiments were compared according to the scheme, which is presented in Table 2. The DNA banding patterns unique and common for resistance sources were isolated. Information on SCN resistance and susceptibility of the genotypes used in RAPD bulk analysis was from studies by Hussey et al., (1991) and Riggs et al., (1988, 1991).

Table 2. Scheme for marker selection in comparison experiment of DNA bulks of SCN resistant and susceptible cultivars with resistance sources.

DNA bulks of cultivars						Resistant Sources			
Race 3		Race 5		Race 14		Essex[c]	PI 88788	Peking	PI 437654
Res.[a]	Sus.[b]	Res.[a]	Sus.[b]	Res.[a]	Sus.[b]				
+	-					-	+	+	+
-	+					+	-	-	-
		+	-			-	-	+	+
		-	+			+	+	-	-
				+	-	-	+	-	+
				-	+	+	-	+	-

[a] DNA bulk of resistant cultivars.
[b] DNA bulk of susceptible cultivars.
[c] Susceptible cultivar.

Results and Discussion

We identified 1622 well resolved RAPD bands from the bulks of soybean cultivars. Eighty two polymorphisms (5.1 % of total number of bands) were detected between DNA bulk of resistant cultivars and DNA bulk of susceptible cultivars to SCN race 3 (Table 3). One hundred sixty three bands (10.0 %) were polymorphic between DNA bulk of resistant cultivars and DNA bulk of susceptible cultivars to SCN race 5. Fifty six polymorphisms (3.4 %) were detected between DNA bulk of resistant cultivars and DNA bulk of susceptible cultivars to SCN race 14 (Table 3).

The comparison of RAPD polymorphic patterns between DNA bulks of cultivars and sources for resistance (Table 2) showed a total of 66 polymorphisms common for the resistant plant introductions and the bulks of resistant cultivars (Table 4). Thirty-nine of these polymorphisms were detected in race 3 resistant genotypes, 20 in race 5 resistant genotypes, and 7 in race 14 resistant genotypes.

The number of RAPD polymorphisms unique to a specific resistant sources is given in Table 4. No Peking-specific bands were detected for SCN race 14 resistance. Peking is susceptible to race 14. The RAPD banding pattern of Peking was the same as that of cv. Essex, but different from banding patterns in P.I. 88788 and P.I. 437654 and the bulk of SCN race 14 resistant cultivars (Table 4). No P.I. 88788-specific bands

were detected for SCN race 5 in the RAPD analysis. P.I. 88788 is susceptible to race 5. The RAPD banding pattern of P.I. 88788 was the same as cv. Essex, but different from that of Peking and P.I. 437654 and the bulk of resistant cultivars to SCN race 5. Only P.I. 437654 (resistant to all known SCN races) had at least one genotype-specific DNA polymorphism for each of races 3, 5 and 14 (Table 4).

Table 3. RAPD polymorphisms between DNA bulks of resistant and sijsceptible cultivars to soybean cyst nematode races 3, 5, and 14.

Total number of primers tested :	192	
Total number of bands generated by primers:	1, 622	
Number of polymorphic primers:	Number	Percentage
Bulks for SCN race 3:	60	31.25
Bulks for SCN race 5:	97	50.52
Bulks for SCN race 14:	52	27.08
Number of polymorphic bands:		
Bulks for SCN race 3:	82	5.06
Bulks for SCN race 5:	163	10.0
Bulks for SCN race 14:	56	3.58

A total of 50 RAPD polymorphisms were observed in the comparison experiment with P.I. 437654. Five polymorphisms were unique to P.I. 437654 and not represented in Peking and P.I. 88788 (Table 4).

Common RAPD banding patterns were observed for the resistance sources and the bulks of cultivars that matched with SCN resistance in these genotypes. P.I. 437654 and P.I. 88788 (both resistant to SCN races 3 and 14) had common banding patterns for races 3 and 14 with the bulks of resistant to race 3 and 14 cultivars (2 bands for each race). P.I. 437654 and Peking (both resistant to SCN races 3 and 5) and the bulks of race 3 and 5 resistant cultivars had 5 and 11 common polymorphisms for race 3 and 5, respectively. P.I. 437654 and Peking and P.I. 88788 (all resistant to SCN race 3) had 25 common polymorphisms with the bulk of race 3 resistant cultivars. The polymorphisms detected from this experiment were employed to facilitate mapping of chromosomal regions for SCN resistance in the cross of Essex x P.I.

437654 (Choi and Skorupska, 1995a, 1995b).

Table 4. RAPD polymorphisms in resistance sources and DNA bulks of resistant and susceptible cultivars to soybean cyst nematode races 3, 5, and 14.

Pattern	Race 3	Race 5	Race 14	Total
PI88788-Specific	2		2	4
Peking-Specific	1	8		9
PI437654-Specific	I	1	3	5
Common to P188788 and Peking	3			3
Common to P.I.88788 and P.I.437654	2		2	4
Common to Peking and P.I.437654	5	1 1		16
Common to P.I.88788, Peking, and P.I.437654	25			25
Total	39	20	7	66

References

Choi, I.S. and H.T. Skorupska. 1995a. Random amplified polymorphic DNA map for intraspecific cross of Essex x P.I. 437654 in soybean, Glycine max (L.) Merr., (In submission).

Choi, I.S. and H.T. Skorupska. 1995b. Detection of molecular markers for resistance to Heterodera glycines, I., races 3, 5, 14, in Essex x P.I. 437654 cross. Crop Sci., (In submission).

Giovannoni, J.J., R.A. Wing, M.W. Ganal, and S.D. Tanksley. 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res. 19:6553-6558.

Hussey, R.S., H.R. Boerma, P.L. Raymer, and B.M. Luzzi. 1991. Resistance in soybean cultivars from maturity groups V-VIII to soybean cyst and root-knot nematodes. J. Nematology. 23:576-583.

Keim, P., T. Olson, and R.C. Shoemaker. 1988. A rapid protocol for isolating soybeanDNA. Soy. Genet. Newsl. 15:151-152.

Mitchelmore, R. W., I. Paran, and R.V. Kesseli. 199 1. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to

detect markers in specific genomic regions using segregating populations. PNAS. 88:9828-9832.

Reiter, R. S., J.G.K. Williams, K.A. Feldman, J.A. Rafalski, S.V. Tingy, and P.A. Scolnik. 1992. Global and local genome mapping in Arabidopsis thaliana by using RIL and RAPD. Proc. Natl. Acad. Sci. 89:1477-1481.

Riggs, R.D., M.L. Harnblen, and L. Rakes. 1988. Resistance in commercial soybean cultivars to six races of Heterodera glycines and to Meloidogyne incognita. Annals of Applied Nematology. 2:70-76.

Riggs, R.D., L. Rakes, and R.Elkins. 1991. Soybean cultivars resistance and susceptible to Heterodera glycines. Supplement to Journal of Nematology 23:5 84-592.

Rogers, S. 0. and A.G. Bendich. 1985. Extraction of DNA from milligrain amount of fresh, herbarium and mummified plant tissue. Plant Mol. Biol. 5:69-76.

Skorupska, H.T., I.S. Choi, A.P. Rao-Arelli, and W.C. Bridges. 1994. Resistance to SCN and molecular polymorphism in various sources of Peking soybean. Euphytica. 75:63-70.

Wang, G. L. and A.H. Paterson. 1994. Assessment of DNA pooling strategies for mapping of QTLS. Theor. Appl. Genet. 88:355-361.

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Molecular phylogeny as a tool for soybean breeding IV

Lark et al., (1992; 1993) and Terry et al., (1994) reported using Rapidly Amplified Polymorphic DNA (RAPD) to establish soybean phylogeny relative to perennial and annual Glycine species. The results were obtained by an undergraduate research class, Molecular Evolution Laboratory, at the University of Utah. In the Fall of 1994, students in the class used RAPDs to resolve several questions from previous years' results. The objectives were: 1) to determine the relationship of Neonotonia wightii, formerly Glycine wightii, to representative taxa of the subtribe Glycininae; 2) to determine relationships of several G. gracilis (alleged hybrid of G. max and G. soja) accessions to Glycine annuals and perennials; and 3) to track parent- progeny relatedness within specific crosses of the pedigree of Essex. Consensus trees of bootstrap analyses were derived to determine the robustness of phylogenies that included sseveral genera and subtribes within the Tribe Phaseoleae.

Materials and Methods

Seeds of perennial species were obtained from William Davis, Ring Around Seed Co. Seeds of annual Glycine spp. were obtained from either K. G. Lark, University of Utah or R. Nelson and C. Coble at the USDA, ARS Soybean Germplasm collection at Urbana. Non-Glycine genera were obtained from either T. Hymowitz at the University of Illinois or S. Kresovich at the USDA, ARS/ University of Georgia, Griffin, Plant Genetic Resources Conservation Unit.

DNA was isolated and RAPD markers were determined using similar procedures described in Lark et al., (1992; 1993). In summary, isolated DNA was used as a template in PCR reactions with 30 different 10mer primers, and the products were separated on agarose gels. Bands on gels, visualized with ethidium bromide, were captured onto a MACIIci computer through a camera using NIH Image 1.56 program. Images were enhanced to clarify banding patterns and then scored relative to standard Kb ladders. The characters were specific size fragments (bands) resulting

from a specific primer PCR. Binary character states were assigned: taxa were labeled as either having (1) or being absent of (0) each particular-sized fragment for each primer. The clarity of each primer's banding pattern was rated on a 0 - 3 scale (0, very difficult to see and score bands and 3, clearly defined bands). These ratings were used as the basis for eliminating characters to test their effect on the stability of phylogenies.

Two sets of plants (27 each) were tested with each primer and each set was run and scored as a separate gel. Gel data set one consisted of Glycine species (annuals, i.e., G. max and G. soja; and perennials) except Neonotonia wightii, which was used as the outgroup. Gel data set two consisted of several annual and perennial Glycine species (subtribe Glycininae), several non-Glycine Glycininae, two species each from subtribes Phaseolinae and Cajaninae, all within the tribe Phaseoleae. Because of the evidence in favor of an early divergence of Cajaninae from other subtribes of the tribe Phaseoleae (Doyle and Doyle 1993), Cajanus cajan was used as the outgroup in gel set two.

We used local optimization searches with heuristic analyses within Phylogenetic Analysis Using Parsimony (PAUP 3.1.1) (Swofford 1993) on Apple Macintosh LC 520 or Quadra 840 computers to produce phylogenies of more than 12 taxa. Robustness of a phylogeny was determined using bootstrap methods of random heuristic searches that produced a 50% Majority Rule consensus tree. The bootstrap technique randomly samples the character set with replacement and gives an estimate of the reliability at each node of the consensus tree (Swofford and Olsen 1990). Exact searches, which find global rather than local optimum solutions, of up to 12 taxa were accomplished using a Branch and Bound search with an upper bound on the tree length. This upper bound greatly reduced the time required to complete the search. The upper bound was determined by running Branch Swapping Heuristic searches (100 replicates each) using the Random Addition Sequence option for starting trees. To compare relatedness among taxa, we examined character distances (which represent absolute distances in character states between taxa) and/or patristic distances (which include homoplasies; i.e., reversals, convergences) represented by the phylogram derived from the parsimony analysis.

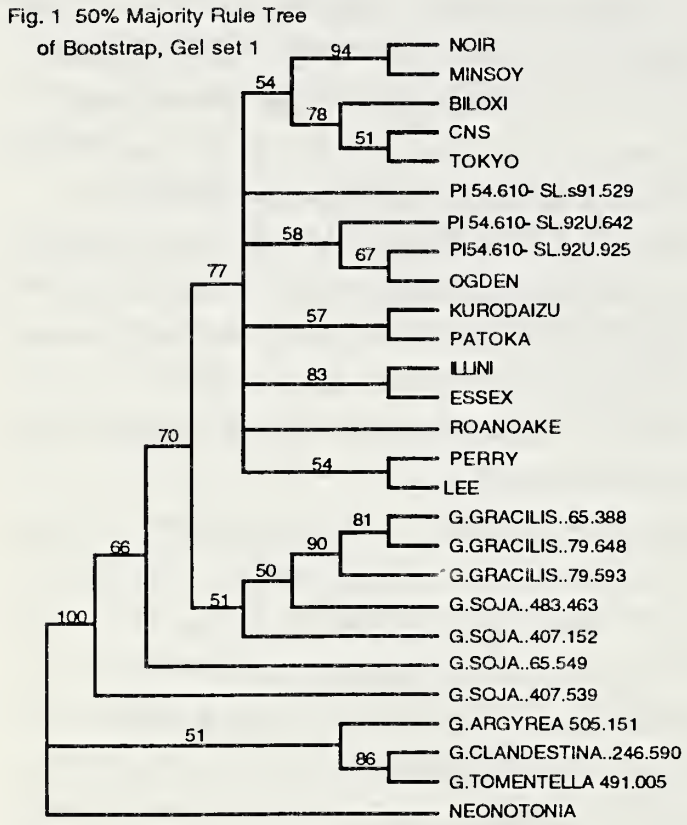
Results and Discussion

Robustness of overall phylogenetic patterns: A preliminary bootstrap analysis,

which included all characters (all 30 primers), resulted in many unresolved branches (those branches with less than 50% reliability) on both gel data sets (data not shown). By eliminating the characters of primers whose gels were hard to score (i.e., those with a rating < 1.0), we increased the stability in tree topology. On the first gel data set (Glycine data set), there were only two unresolved branches (Fig. 1). The Glycine perennials separated from the annual species, G. max and G. soja, in 100% of the bootstraps. G. soja 483.463 and 407.152 were located within the G. gracilis subclade. Many relationships were similar to the expected but with less than 100% reliability. With the second gel data set containing a more diverse taxa, there were several unresolved branches (Fig. 2). This may be due to a constraint of RAPDs to effectively resolve such diverse taxa (Clark and Lanigan 1993). Nevertheless, some interesting trends resulted. As in the first gel set, the Glycine annuals separated from the rest of the taxa in 100 % of the bootstraps indicating a monophyletic origin. Also, Cajanus spp. separated from all other taxa in 100% of the bootstraps. There was no clear separation of G. soja from G. max, but the four G. max appeared to be derived from the G. soja. The two Amphicarpa species always formed a subclade and two of the Teramnus species were sister taxa in 63% of the bootstraps. One contrast between the first and second gel data sets was the relationship among three of the G. max. Noir and Minsoy formed a tight subclade in gel set one (Fig. 1), but CNS and Noir formed a subclade 98% of the time in the second gel data set (Fig. 2). This contrast could be due to different taxa in each gel set influencing how G. max link to each other or due to missing lanes of specific cultivars between the gel sets.

Relationships among representative Phaseolinae, Cajaninae and non- Glycine Glycininae taxa: The Glycine species were removed from this analysis to use exact methods to resolve positions among the 12 non-Glycine genera of gel set two. The Branch and Bound exact search of PAUP produced two equally parsimonious trees(Fig. 3A, B). Except for the disjunction between the two Pueraria species, one of the solutions, Fig. 3A, conforms to Lackey's 1981 classification. If this tree represents evolutionary history then P. phaseoloides has a basal placement to the Phaseolinae and P. lobata has a basal placement to the remaining Glycininae. The genus Pueraria, composed of at least three distinct groups, is considered a "collecting site" for hard to place Glycininae (Lackey 1981). The two species we analyzed represent two of the groups. Although both Pueraria spp. had similar character distances to each genus or species within the Glycininae, P. phaseoloides was much closer to Phaseolinae and Cajaninae than the Pueraria were to each other. The species name "phaseoloides" means "phaseol-like". The phylogeny in Fig. 3B contrasts with that of

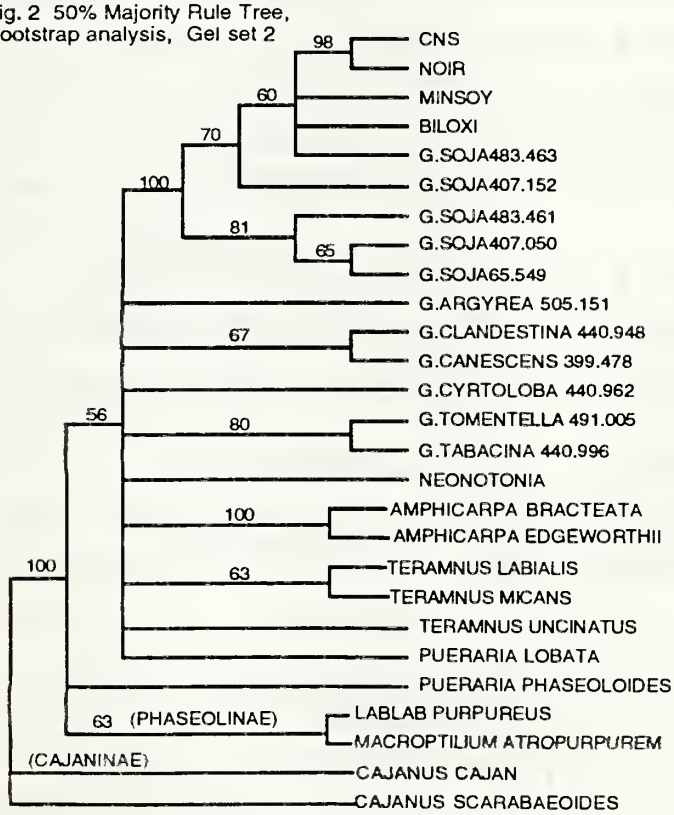
Fig. 3A by the separation of Teramnus uncinatus from the other two species of this genus. If information from both these trees is coupled with chromosome and morphological data, the phylogeny of Fig. 3A is the most parsimonious because chromosome number within the Teramnus (2n=28) contrasts with all other Glycininae (2n=22) except for Glycine spp., (2n=40 or 80) (Kumar and Hymowitz 1989), and the Teramnus spp. are the only ones that share a similar anomalous style and alternate abortion of anthers (Lackey 1981). If Fig. 3B phylogram is the one that reflects evolutionary history, the change in chromosome number and the anomalous morphology would have arisen at least twice which is less likely than the monophyletic origin indicated in Fig. 3A.



Position of Neonotonia wightii, formerly G. wightii, among Glycininae: Neonotonia wightii was removed from the Glycine genus in 1977 (see Lackey 1981) based on contrasting chromosome size and number (2n=22, 44) and morphology with

the Glycine genus. In last year's data set (Terry et al. 1994), Neonotonia was closer to the genus Glycine (particularly G. argyrea) than it was to the non-Glycine Glycininae tested, possibly questioning the removal of the Neonotonia from the Glycine genus. This year's data set included additional genera and species in the subtribe Glycininae. Neonotonia was approximately equidistant to non-Glycine Glycininae (234 avG. character distance) and Glycine perennial species (232) and was more distant to Glycine annuals (242), Cajaninae (245) and Phaseolinae (250) (also see Fig. 2). In addition, the character distance between species within most genera (163 avg. character distance) (excluding Pueraria and Glycine perennials with annuals) was less than Neonotonia with any other taxon (lowest character distance was 219). This supports the placement of Neonotonia into its own genus.

Fig. 2 50% Majority Rule Tree,
Bootstrap analysis, Gel set 2



Glycine gracilis, the weedy hybrid of domesticated (G. max) and wild (G. soja) soybean?

The two G. gracilis accessions analyzed by Terry et al., (1994) varied in their

relationships to Glycine species and the difference was in part due to the similarity of G. soja 483.463 to G. gracilis 65.388, one of the accessions. However, both accessions could not be compared directly because each one was on a separate gel. The three G. gracilis accessions analyzed in the current study were all on the same gel and formed a tight subclade within the annual Glycine species (90% reliability) (Fig. 1). The character distance between G. gracilis 65.388 and 79.648 was 34, the lowest distance between any two taxa in the data set. G. soja 483.463 was approximately equidistant to G. gracilis (101-109 character distance) and G. soja (105-117). Each G. gracilis was slightly closer to the rest of the G. soja (avg., 123 character distance) than to the G. max (134). All three G. gracilis were much closer to the Glycine annuals than they were to the Glycine perennials (238 avG. character distance). This supports the hypothesis that G. gracilis is a hybrid of G. soja and G. max (Lackey 1981). The similarity of G. soja 483.463 to G. gracilis suggests that it is closely related to the G. soja parent involved in the cross.

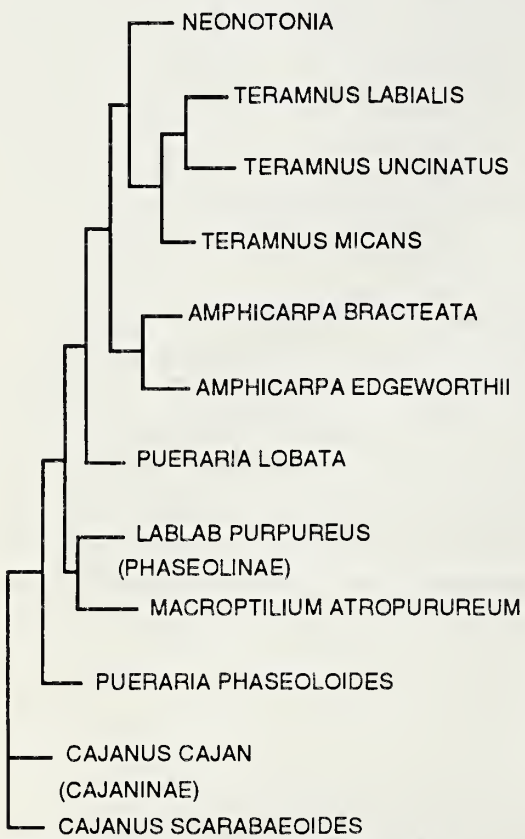


Fig. 3A SOLUTION 1, NON-GLYCINE TAXA

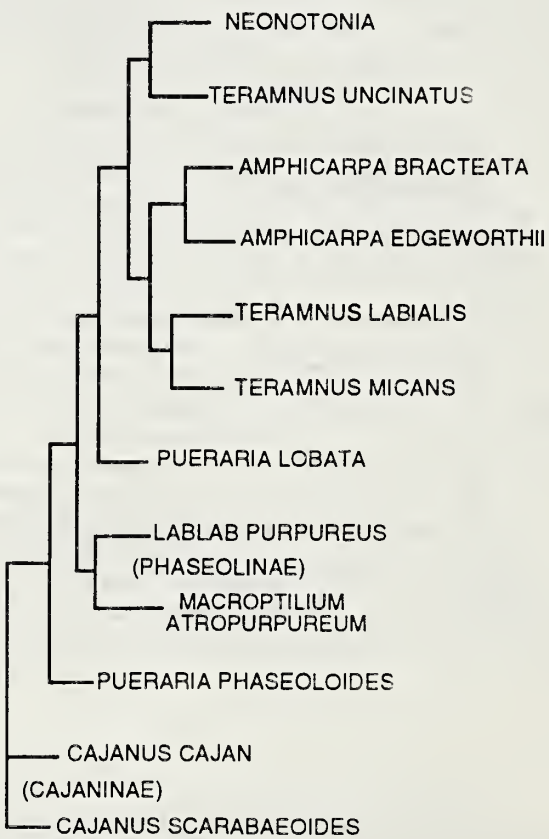


FIG. 3B SOLUTION 2

Relatedness among cultivars within the Essex pedigree

Past years' results (Lark et al., 1993; Terry et al., 1994) have indicated that the relatedness of Essex to cultivars involved in its pedigree did not agree with potential relatedness (i.e, 100-(% of parental genome expected)), probably due to selection by breeders during the development of the cultivars. This year's data set included several additional lines within the pedigree of Essex to determine % similarity of characters (100 X (total characters-character distance)/total characters) across specific parent -progeny lines. Overall, we found that character states of 51% of the characters (274 of 543) did not vary across the 11 cultivars analyzed. The average % similarity among pairs of PI lines, that are presumably independent lines, was 80%. In almost every case presented below, the resulting cultivar had a much lower character distance to one parent of the cross than the other indicating the direction of the selection.

1) Ogden (cross of Tokyo , P.I. 8.424, X P.I. 54.610). Of the three sublines (SL) of P.I. 54.610 tested, SL 92U.925 was much closer (48 character distance) to Ogden than SL 92U.642 or SL s91.529 (63 and 100 character distance, respectively). All three P.I. 54.610 SL were closer to Ogden than Tokyo was to Ogden (125 character distance) (Fig. 1).

2) Perry (cross of Patoka, P.I. 70.218, X a rogue of Kurodaizu, P.I. 81.041). Perry was closest to Lee (not part of the cross) (67 character distance), and then Patoka (80 characters). Kurodaizu had the highest character distance (109) of any taxon to Perry.

3) Lee (CNS, P.I. 71.597, X Illini). Lee was closet to Perry (see above) and was slightly closer to Roanoke (a rogue of CNS) (98 character distance) than it was to CNS (104 characters) or Illini (109 characters). Lee was farthest from Kurodaizu (120 characters).

4) The % potential relatedness of each line to Essex (Table 1) was compared with the observed % similarity and character distance. The observed similarity was very high because it included those characters (51%) that did not vary across the cultivars. There was some agreement between the potential and observed in the direction of the similarity (i.e., of those predicted to be more similar to Essex, Illini was the most similar and, as predicted, Tokyo, Ogden and 54.610 were the most distant). The most notable contrast was that of CNS, which was much lower than expected. This result has been consistently observed in our earlier data sets. CNS is widely used in crosses because of its bacterial pustule resistance gene.

Table 1. The % potential relatedness of cultivars to Essex based on Mendelian segregation ratios of the pedigree compared with the % observed similarity and character distances.

Cultivar	% Potential	% Observed similarity	Character distance
Lee	50	81	107
CNS	44	79	121
Illini	25	91	56
Perry	25	85	84
Patoka	13	83	92
Roanoke	13	85	97
Kurodaizu	13	80	111
Ogden	6	76	133
PI 54.610	3	76	135
Tokyo	3	78	124

References

Clark, A.C. and C.M.S. Lanigan. 1993. Prospects for estimating nucleotide divergence with RAPDs. Mol. Biol. Evol. 10: 1096-1111.

Doyle, J.J. and J.L. Doyle 1993. Chloroplast DNA phylogeny of the Papilionoid Legume Tribe Phaseoleae. Syst. Bot. 18:309-327.

Kumar, P. and T. Hymowitz 1989. Where are the diploid (2n= 2x= 20) genome donors of Glycine Willd., (Leguminosae, Papilionoideae). Euphytica 40: 221-226.

Lackey, J.A. 1981. Phaseoleae DC. pp. 301-327 In: Polhill, R. M. and P. H. Raven (eds). Advances in Legume Systematics. Kew: Royal Botanical Gardens.

Lark, K.G., J. Evans, F. Basha, R. Bogden, R. Copeland, R. Ellison, D. Horne, K. Lee, K. McDonald, J. Pierson, W. Schuster, P. Wilhelm and Y. Yu. 1992. Molecular Phylogeny as a tool for soybean breeding. Soybean Genetics Newsletter. 19:174-181.

Lark, K.G. , J. Evans, P. Wilhelm, S. Atkinson, M. Christensen, B. Crockett, B. Dunford, C. Jones, S. Mendenhall, T. Messick, C. Prout, P.E. Solomon, M. Stevens, I.

Terry, C. Walker, C.L. Wong, R. Ellison. 1993. Molecular Phylogeny as a tool for soybean breeding II. Soybean Genetics Newsletter 20: 197-201.

Swofford, D.L. and G. J. Olsen. 1990. Phylogeny reconstruction. pp.411-501 In: Hillis, D M. and C. Mortis (eds) Molecular Systematics. Signer Press, Sunderland, MA.

Swofford, D.L. 1993. PAUP version 3.1.1. Illinois Natural History Survey, Champaign, Ill.

Terry, I., K.G. Lark, L. Allphin, M. Stevens, A.J. Angilau, M. Gay, L. Harrison, B. Huff, P. Magasich, G. Miller, G. Nishimoto, C. Rhead, Q. Sahratian and J. Whitehead, J. A. Whitehead. 1994. Molecular Phylogeny as a tool for soybean breeding III. Soybean Genetics Newsletter. 21: 245-256.

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Inheritance of an unevenly distributed brown seed coat pigmentation pattern in soybean

A seed coat pigmentation off-type was recently identified in a seed increase of Crockett soybean. The brown seed coat pigmentation pattern is unusual in that the coloration is expressed strongly in a saddle pattern and also, to a lesser degree, in the remainder of the seed coat. When the seeds were planted the following growing season, plants exhibited a Crockett phenotype (Bowers, 1990) for all observable characters except seed coat pigmentation. Seed samples were sent to workers at the USDA soybean germplasm collection (Urbana, Illinois) but, the pigmentation pattern could not be distinctly characterized. Therefore, an inheritance study was undertaken to determine genetic control of the trait.

Materials and methods

In 1993, the brown Crockett seed coat strain identified as Crockett-br, was used in five different crosses with four other genotypes of contrasting seed coat and hilum pigmentation characteristics. The parents and their phenotypes are presented in Table 1. F_1 seed were grown in Puerto Rico the following winter and F_2 populations were grown at the Simpson Agricultural Experiment Station (Pendleton, SC) in 1994. Five F_2 populations were scored on an individual plant basis for coloration of seed coat and hilum. Segregation of seed coat pigmentation patterns were evaluated for expected to observed genetic ratios using chi-square analyses.

Results and Discussion

In reciprocal crosses with normal Crockett, 3:1 segregation patterns were observed with yellow seed coat being dominant to brown (Table 1). Segregation at the R locus was indicated by 3:1 segregation in the Maxcy-bl cross with black being

dominant to brown (Table 2). Dominance of yellow to brown and black to brown have been reported previously (Williams, 1951: Bhatt and Torrie, 1968). Verification of segregation at the i locus with classical dominance effects for hilum and seed coat patterns were indicated by crosses with Hagood and Perrin (Table 2). Furthermore, all of the self black and self buff seed coat segregates in crosses with Maxcy-bl, Hagood, and Perrin exhibited a consistent level of pigmentation throughout the seed coat indicating that there was no segregation at the i^k locus. Seed coat scores for Crockett-br x Hagood resulted in imperfect black segregates and black segregates being combined in the same phenotypic class. The unexpected presence of black/imperfect black segregates in the Crockett-br x Hagood F_2 population suggest that Hagood soybean may have imperfect black hilum color rather than buff as described previously.

Table 1. Description of seed coat patterns and hilum color of parents used for inheritance study

	Phenotype color			
Parent	Flower	Pubescence	Seed coat	Hilum
Crockett	Purple	Tawny	Yellow	Brown
Crockett-br	Purple	Tawny	Brown	Self
Maxcy-bla	Purple	Tawny	Black	Self
Hagood	White	Gray	Yellow	Imperfect black[b]
Perrin	Purple	Tawny	Yellow	Black

[a] A self black seed coat off-type found in Maxcy soybean.
[b] Based on results from this study. Previously described as buff.

Conclusion

Our data indicates that the atypical self seed coat pigmentation pattern in Crockett-br is a mutation from i^i to i. Mutations from i^i to i tend to be relatively common but, generally give a more even distribution of pigment throughout the seed coat. We report a soybean self seed coat mutation from i^i to i that gives a dark brown saddle pattern and light brown pigmentation in the remainder of the seed coat. Seed samples of Crockett-br may be obtained by contacting Jeff P. Tomkins or Emerson R. Shipe (Clemson University).

Table 2. F_2 segregation of seed coat pattern and hilum color of five soybean crosses.

	Seed coat color							
	Yellow			Brown	Black[a]	Buff		
	Hilum color							
Cross	Brown	Black[a]	Buff	Self	Self	Self	χ^{2*}	Expected F_2 ratio
Crockett x Crockett-br	115	-	-	40	-	-	0.05	3:1
Crockett-br x Crockett	95	-	-	24	-	-	1.48	3:1
Maxcy-bl x Crockett-br	-	-	-	49	159	-	0.06	3:1
Hagood x Crockett-br	39	81	38	12	24	15	5.50	3:1 x 1:2:1
Perrin x Crockett-br	63	169	-	13	59	-	2.62	3:1 x 3:1

* All χ^2 values < 5% tabular values indicating satisfactory fits of observed to expected values.

[a] For Hagood x Crockett-br, imperfect black and black segregates combined in same class.

References

Bhatt, G.M. and J.H. Torrie. 1968. Inheritance of pigment color in the soybean. Crop Sci. 86:17-619.

Bowers, G.R. 1990. Registration of 'Crockett' soybean. Crop Sci. 30:427.

Wiiliams, L.F. 1952. The inheritance of certain black and brown pigments in the soybean. Genetics 37:208-215.

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Influence of cytokinin on photomorphogenic development associated with the floral transition in soybean

Characteristics of growth and subsequent floral development in response to cytokinin applied to soybeans during vegetative growth is not well documented. During the vegetative phase of soybean development, endogenous cytokinin levels and branch development are suppressed (Heindl et al., 1982; Carlson et al., 1987). When the soybean plant enters the reproductive phase, endogenous cytokinin levels and branch development increase (Board and Settimi, 1986; Heindl et al., 1982; Carlson et al., 1987). Altering soybean plant development with cytokinin applied during vegetative growth when endogenous levels are low may influence subsequent reproductive morphogenesis by providing more sites for floral structures to develop. Therefore, a two part study using growth chamber and greenhouse environments was undertaken. The overall goal was to examine growth and developmental responses of soybeans grown under inductive photoperiods to cytokinin (benzylaminopurine) applied during two different stages of vegetative growth. In addition, isogenic soybean genotypes having either a conventional or delayed flowering response trait were utilized to determine the influence of cytokinins on two different floral genetic types.

Materials and methods

In the growth chamber study, two isogenic pairs; F85-1138 / F85-1136 and F85-8099 / F85-8089 were established in 1000 ml pots containing a Cecil sandy loam soil (clayey, kaolinitic, thermic Typic Hapludults). Plants were thinned to 2 uniform plants pot^{-1} after emergence. The experiment was conducted under a constant 10-h photoperiod with metal halide light sources to provide a photosynthetic photon flux density of 450 $\mu mol\ m^{-2}\ s^{-1}$ at the top of the plant canopy. Growth chamber temperature was maintained at 27 / 16 °C during the day/night cycle. Benzylaminopurine (BA) treatments were applied to one group (BA-early) of plants at

the Ve and V1 stages and to the second group (BA-late) at the V_2 and V_3 stages (Fehr et al., 1971). Control groups received the carrier solution in the same manner as the BA treatment groups. Cytokinin treatment consisted of a 3 ml solution of BA at 2000 µM (Crosby et al., 1981) applied in a foliar mist to each individual plant with a hand held spray atomizer. BA was dissolved in a carrier of acidified H_2O (pH 5.5) and 4% dimethylsulfoxide (DMSO). Beginning at emergence, vegetative growth stages were recorded over the course of the study to determine growth rate. At the R_2 growth stage, plants were harvested and divided into shoot (above cotyledonary node) and root (below cotyledonary node). Shoots were separated into stem and leaf fractions. Leaf area was measured using an area meter and main stem length, number of nodes, total branch length, number of branches, number of floral buds, and number of floral nodes were determined. Leaf and stem were combined and plant material was dried at 60 °C for 48 h and dry matter weight for shoot and root fractions were determined.

Upon completion of the growth chamber study, a greenhouse experiment was conducted. The 2 isogenic pairs used previously and one additional pair F85-8296 / F85-8293 were established in a Riverview loam (fine-loamy, mixed, thermic Fluventic Dystrochrepts). At emergence (22 March) the photoperiod (sunrise to sunset) was 12.2 h and increased at a rate of ~2 min d^{-1}. Greenhouse temperature was maintained at 27/16 °C during the day/night cycle. BA treatments were applied to one group at the Ve and V1 stages and to the second group at the V_2 and V_3 stages. DMSO concentration was reduced to 2% to reduce leaf burning. Experimental designs were CRD factorials with 4 and 6 replications in the growth chamber and greenhouse, respectively.

Results and Discussion

The control responses from early and late treatments containing the carrier solution (no BA) were similar in the growth chamber and greenhouse experiments so the mean of these two treatments is referred to as the control. In both environments, BA treatments did not affect the rate of V-stage development (measured in days after emergence) compared to the control ($P < 0.05$). Also, the rate of vegetative development was similar within isogenic pairs. Delayed flowering genotypes exhibited extended vegetative development in both experiments compared to conventional genotypes. BA treatment, however, did not influence the length of the vegetative cycle for either floral type compared to the control (data not shown). Average flowering delay within isogenic pairs was 6 d in the growth chamber and 10 d in the greenhouse.

Floral morphology was altered with BA treatment in both experiments with the greatest response being observed for the late BA treatment (Table 1) giving an average 26 and 27% increase across experiments in the number of floral buds and nodes, respectively, compared to the control. For main stem length, early BA treatment had a stimulatory effect on one line in the growth chamber while other genotypes remained unaffected (Table 2). Late BA treatment had no stimulatory effect on main stem length in either experiment. Total branch length, however, was increased an average 37% across experiments over the control (Table 2). Number of nodes and branches were increased an average 20 and 50%, respectively over the control (Table 3). In the greenhouse, delayed flowering genotype node and branch morphology were not stimulated by late BA treatment but, were actually suppressed by early BA treatment. Changes in leaf area in response to BA treatment were not detected (Table 4). Increases in shoot and root dry matter were generally not detected except for F85-1136 which showed an increase in shoot dry matter in the growth chamber and an increase in root dry matter in the greenhouse in response to late and early BA treatment, respectively.

Table 1. Soybean floral morphology in response to cytokinin.

	No. floral buds				No. floral nodes			
Experiment/ isogenic pair	Control	BA-early	BA-late	LSD[a]	Control	BA-early	BA-late	LSD[a]
Growth Chamber	plant [1]							
F85-1138*	26.3	29.3	29.5	NS	6.8	6.8	7.5	NS
F85-1136	12.4	15.0	21.3	4.0	3.9	5.3	9.0	1.4
F85-8099*	18.6	23.8	28.5	8.4	6.0	7.0	9.0	1.9
F85-8089	16.4	27.5	25.5	8.7	5.8	8.0	8.3	2.0
Greenhouse								
F85-1138*	27.2	20.0	26.3	6.2	8.7	4.8	8.3	1.7
F85-1136	14.9	17.7	28.2	3.8	4.2	5.0	7.2	1.4
F85-8296*	31.2	28.2	29.0	NS	7.5	6.2	7.0	1.2
F85-8293	16.6	18.5	25.3	6.1	5.1	4.8	7.3	1.1
F85-8099*	26.1	24.8	24.2	NS	8.6	9.5	8.8	NS
F85-8089	20.4	25.7	30.7	6.8	8.2	9.2	11.6	2.5

* Delayed flowering time
[a] 0.05 level

Table 2. Soybean branch and stem growth in response to cytokinin.

Experiment/ isogenic pair	Main stem length				Total Branch length			
	Control	BA-early	BA-late	LSD[a]	Control	BA-early	BA-late	LSD[a]
Growth Chamber	mm plant [1]							
F85-1138*	333	393	248	73	33	33	34	NS
F85-1136	185	243	170	NS	4	13	35	22
F85-8099*	351	583	283	200	5	0	16	NS
F85-8089	241	293	223	61	0	9	15	NS
Greenhouse	mm plant [1]							
F85-1138*	137	125	142	NS	40	8	33	28
F85-1136	88	98	95	NS	3	9	18	12
F85-8296*	138	135	135	NS	31	12	24	15
F85-8293	101	112	108	NS	3	0	17	10
F85-8099*	149	155	150	NS	60	65	67	NS
F85-8089	126	128	133	NS	54	40	80	20

* Delayed flowering time
[a] 0.05 level

Table 3. Soybean floral morphology in response to cytokinin.

Experiment/ isogenic pair	No. nodes				No. branches			
	Control	BA-early	BA-late	LSD[a]	Control	BA-early	BA-late	LSD[a]
Growth Chamber	plant [1]							
F85-1138*	11.2	12.5	12.1	NS	1.8	2.3	2.3	NS
F85-1136	6.1	8.0	12.7	1.9	0.4	0.7	2.0	1.2
F85-8099*	9.3	10.8	12.0	2.4	0.4	0.0	1.0	NS
F85-8089	8.0	10.5	10.8	1.5	0.0	0.8	1.3	NS

Table 3. continued

Experiment/ isogenic pair	No. nodes Control	BA-early	BA-late	LSD[a]	No. branches Control	BA-early	BA-late	LSD[a]
Greenhouse				plant [1]				
F85-1138*	10.6	7.7	10.2	2.0	2.4	0.7	2.5	1.3
F85-1136	5.8	6.7	8.3	1.5	0.3	0.8	1.8	1.2
F85-8296*	11.0	8.8	10.8	1.6	1.8	0.8	2.0	0.8
F85-8293	7.2	6.8	8.8	1.5	0.3	0.0	1.0	0.9
F85-8099*	12.6	14.3	13.3	NS	3.2	2.7	3.0	NS
F85-8089	11.3	11.8	14.3	NS	2.5	2.7	3.7	NS

* Delayed flowering time
[a] 0.05 level

Table 4. Soybean floral morphology in response to cytokinin.

Experiment/ isogenic pair	Leaf area Control	BA-early	BA-late	LSD[a]	Shoot dry matter Control	BA-early	BA-late	LSD[a]	Root dry matter Control	BA-early	BA-late	LSD[a]
Growth Chamber		cm2 plant [1]					mg plant [1]					
F85-1138*	175	169	163	NS	119	115	114	NS	66	104	123	NS
F85-1136	107	107	139	NS	62	62	94	NS	32	51	65	NS
F85-8099*	219	236	230	NS	151	185	163	NS	69	84	76	NS
F85-8089	168	197	203	NS	131	164	152	NS	66	84	87	NS
Greenhouse												
F85-1138*	200	168	207	NS	113	98	125	NS	73	78	66	NS
F85-1136	109	113	107	NS	45	43	37	NS	28	88	42	NS
F85-8296*	313	254	280	NS	207	177	197	NS	100	106	105	NS
F85-8293	186	159	171	NS	111	88	108	NS	83	69	61	NS
F85-8099*	252	275	267	NS	177	192	166	NS	95	105	76	NS
F85-8089	198	204	186	NS	132	129	114	NS	78	90	66	NS

* Delayed flowering time
[a] 0.05 level

Conclusion

Foliar cytokinin treatment to photoperiod-induced soybeans during vegetative growth was effective in stimulating developmental changes detected at the floral transition (R_2 stage). Cytokinin treatments during the V_2-V_3 growth stages stimulated more changes in floral, stem, and branch morphology than treatments applied during the V_e-V_1 stages. Vegetative growth rates and length of the vegetative cycle were not altered with BA treatment. Generally, conventional flowering soybean genotypes appeared to be more responsive to BA stimulated morphological changes than delayed flowering types. These experiments were limited in scope and should be considered preliminary and speculative in nature.

References

Board, J.E., and J.R. Settimi. 1986. Photoperiod effect before and after flowering on branch development in determinate soybean. Agron. J. 78:995-1002.

Carlson, D.R., D.J. Dyer, C.D. Cotterman, and R.C. Durley. 1987. The physiological basis for cytokinin induced increases in pod set in IX93-100 soybeans. Plant Physiol. 84:223-239.

Crosby, K.E., L.H. Aung, and G.R. Buss. 1981. Influence of 6-benzylaminopurine on fruit-set and seed development in two soybean, Glycine max (L.) Merr. genotypes. Plant Physiol. 68:985-988.

Dyer, D.J., D.R. Carlson, C.D. Cotterman, J.A. Sikorski, and S.L. Ditson. 1987. Soybean pod set enhancement with synthetic cytokinin analogs. Plant Physiol. 84:240-243.

Fehr, W.R., C.E. Caviness, D.T. Burmood, J.S. Pennington. 1971. Stage of development descriptions for soybeans, Glycine max (L.) Merrill. Crop Sci. 11:929-931.

Heindl, J.C., D.R. Carlson, W.A. Brun, and M.L. Brenner. 1982. Ontogenetic variation of four cytokinins in soybean root pressure exudate. Plant Physiol. 70:1619-1625.

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The soybean cyst nematode resistance gene on linkage group G is common among sources of resistance

To identify genes for soybean cyst nematode resistance, we analyzed four segregating soybean F_2 populations ('Evans' x P.I. 209332, Evans x P.I. 90763, Evans x P.I. 88788 and Evans x 'Peking') and a $F_{5:6}$ nearly-recombinant inbred population derived from the Evans x P.I. 209332 cross.

Among all populations, we identified two to four independent partial resistant loci significantly associated with SCN resistance. One of these loci, located on the top of linkage group G, behaved as a major partial resistance gene and was common in all the populations studied. This locus explained up to 48.6% of total phenotypic variation based on r-square estimates in P.I. 209332, 44.8% in P.I. 90763, 30% in P.I. 88788 and 22.5% in Peking populations.

Materials and Methods

The mapping populations were constructed by crossing Evans with the following sources of SCN resistance: P.I. 209332, P.I. 90763, P.I. 88788, and Peking. In each of the crosses, a single F_1 seed was advanced to the F_2 generation. The 76 to 113 F_2 individuals, together with the parents, were either grown in the greenhouse or in the field and used as source of leaf tissue for DNA extraction and RFLP analysis. Plants were allowed to recover and set F_3 seeds, which were saved for SCN disease assay. The P.I. 209332 population was studied in detail and advanced to the F_5 generation by single seed descent to generate a nearly-recombinant inbred population. For the recombinant inbred population, F_5 plants were row-planted and used as source of leaf material for DNA analysis. F_6 seeds were bulked from each F_5 line for SCN analysis. DNA extraction, restriction digests, electrophoresis, Southern blots, hybridization, and autoradiography were done following conventional methods. (Dellaporta, 1983; Southern, 1975; Young et al., 1992). A total of 10-12 progeny

seedlings for each line were assayed for SCN resistance using the waterbath method (Concibido et al., 1994). Each plant was inoculated three days after germination with 2,000 SCN eggs of a field isolate from Minnesota that behaved as Race 3. Soil temperatures were maintained at 28 °C at 16-hour daylength for 28 days. On the 28th day, individual plants were uprooted and cysts were collected by blasting the roots with pressurized water. The total number of cysts from individual plants were counted under a dissecting microscope and converted to an index by dividing this number by the total number of cysts on the susceptible parent. The strategy used in locating potential partial resistance loci in this study was to detect significant associations between DNA marker genotypes and corresponding SCN disease responses using regression analysis and analysis of variances. A level of significance of $P < 0.002$ (Lander and Botstein, 1989) was chosen to minimize the chances of false positives experiment-wide.

Results and Discussion

Analysis of genetic relationships using classical genetics has revealed that resistance genes to SCN Race 3 may be shared among sources of resistance (Rao Arelli and Anand, 1988). We have demonstrated that a major partial resistance SCN gene for SCN Race 3 on linkage group G (Figure 1) is common between P.I. 209332, P.I. 88788, P.I. 90763 and Peking using restriction fragment length polymorphism (RFLP) analysis. This same region has been found to be significant in P.I. 437654 (D. Webb, Pioneer Hi-Bred, Johnston, IA) and P.I. 88287 (S. Mackenzie, Purdue University, W. Lafayette, IN). This information now provides a basis for our gene deployment strategies in breeding SCN resistant soybeans. Table 1 shows the estimated phenotypic effect on SCN disease response that can be explained by the partial resistance locus on linkage group G in the different populations studied. In the Evans x P.I. 90763 population for instance, homozygotes for the resistant allele on average had a cyst index that was 0.50 (2 x .025) units less than that of homozygous susceptible individuals.

Now that we have identified this major partial SCN resistance locus, efforts are being directed to further characterize and isolate this region. Among the mapping populations, the P.I. 209332 F_2 and RIL populations were the most characterized. Using comparative mapping between Glycine max and Vigna radiata and Phaseolus vulgaris, we have uncovered several Vigna and Phaseolus markers that are tightly

linked with SCN resistance in soybean. This led to an increase in marker density near the SCN resistance gene from one marker every 10 centimorgans to one marker every two centimorgans. Experiments are now underway using pulse field gel electrophoresis of high molecular weight DNA to construct a high resolution map of the G region as a starting point to cloning this major partial resistance gene based on map position.

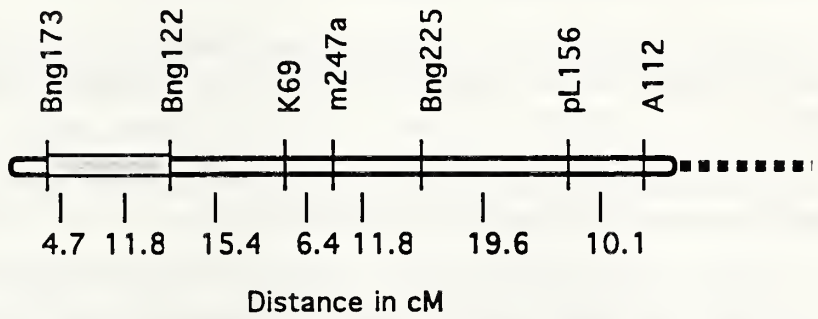

Figure 1. The top of linkage group G where the putative major SCN partial resistance gene is located. Dark region of the chromosome indicates the most likely location of the SCN gene, dashed line indicates the remainder of the linkage group.

Table 1. Partial SCN resistance locus on linkage group G in various mapping populations.

Mapping Population	Percent of total variation	Phenotypic Effect	d/a*	Probability
209332				
209/Evans F_2 population	44.6	-0.20	-0.04	<0.0001
209/Evans RIL population	48.6	-0.18	-0.03	<0.0001
90763	44.8	-0.25	-0.08	<0.0001
88788	30.0	-0.17	-0.23	<0.0001
'Peking'	22.5	-0.16	-0.58	<0.0001

*-The ratio of dominance to additivity, 0 indicates complete additivity, 1 indicates completely dominant, -1 indicates completely recessive.

References

Dellaporta SL, J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: version II. Pl. Mol. Biol. Rep. 1:19-21.

Concibido V.C., R.L. Denny, S.R. Boutin, R. Hautea, J.H. Orf, and N.D. Young. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (Heterodera glycines Ichinohe). Crop Sci 34:240-246.

Lander E.S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185.

Rao-Arelli A.P. and S.C. Anand. 1988. Genetic relationships among soybean plant introductions for resistance to race 3 of soybean cyst nematode. Crop Sci. 28:650-652.

Southern E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Young N.D., L. Kumar, D. Menancio-Hautea, D. Danesh, N.S. Talekar, S. Shanmugasundarum, and D.H. Kim. 1992. RFLP mapping of a major bruchid resistance gene in mungbean Vigna radiata (L.) Wilczek. Theor. Appl. Genet. 84:839-844.

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Physical mapping of DNA markers near a major soybean cyst nematode resistance locus

Genetic mapping based on DNA markers has progressed in soybean to the point that a medium density map with more than 400 DNA markers is now available (R. Shoemaker, Iowa State University, personal communication). With this map, genes controlling resistance to several disease resistance loci, including Phytophthora and soybean cyst nematode (SCN), have been located in terms of nearby marker loci (Concibido et al., 1994; Diers et al., 1992). Thus, it is now possible to construct genetic linkage maps composed of numerous marker loci around any target gene of interest. In genetic linkage mapping, the distance between markers is based on the frequency of recombination events in a defined population. Physical mapping of high molecular weight (HMW) DNA digested with rare cutting restriction enzymes and separated by pulse field gel electrophoresis (PFGE) provides a strategy for determining the actual number of nucleotides separating two DNA sequences. In contrast to genetic mapping, physical mapping does not depend on the presence of fragment length or primer site polymorphisms. Physical mapping sometimes makes it possible to determine the precise order of DNA markers even if there are no recombinants among them. Finally, physical mapping provides an essential basis for efforts at map-based gene cloning.

To study a major SCN resistance locus in soybean, a genetic linkage map was constructed by crossing 'Evans' (susceptible) and P. I. 209332 (resistant) soybeans. RFLP mapping of SCN resistance in an F_5/F_6 recombinant inbred population uncovered a region on linkage group G that was highly correlated with SCN resistance (Concibido et al., 1995, in preparation). Since there was a distance of nearly 20 centimorgans (cM) among the markers spanning this region, comparative genetic mapping with soybean relatives, Vigna and Phaseolus, was employed to increase marker density (Boutin, et al., 1995). This led to an increase in marker density near the SCN resistance gene to one DNA marker every two cM. Physical

based on PFGE analysis was then used to dissect the linkage among the markers mapping near the SCN resistance locus on chromosome G.

Materials and Methods

Preparation of HMW DNA: Young fully expanded leaves of soybean cv. 'Faribault' were harvested and ground to powder in liquid nitrogen. Ten grams of frozen tissue were homogenized on a magnetic stirrer with 60 ml cold extraction buffer (3 mM Spermidine; 1 mM Spermine ; 10 mM Na2EDTA; 10 mM Tris; 160 mM NaCl; 600 mM D-mannitol; 0.1%, w:v Triton X-100; 0.1%, w:v BSA (fraction V); 10 mM ß-mercaptoethanol; pH 9.3-9.5 with NaOH) for 8-10 minutes on ice. The suspension was gently strained through two layers of cheese cloth and one layer Miracloth and then centrifuged at 700-900 x g for 15 min. The pellet was re-suspended in 15 ml extraction buffer and re-precipitated. This washing was repeated two times. The final pellet was re-suspended in an equal volume of extraction buffer without mercaptoethanol and Triton X-100, pH 9, then warmed to 45 °C in a water bath for 5-10 minutes and mixed with equal volume of pre-warmed (45 °C), 1.5% low melting point agarose in extraction buffer without mercaptoethanol and Triton X-100, pH 9. At this point, the mixture was transferred into pre-cooled plug molds and placed at 4 °C for 10-15 min. to solidify the agarose. The agarose inserts were then transferred into 3 volumes of ESP (0.5M EDTA pH 9.3, 1% sodium N-lauroyl Sarkosine and 0.5 mg/ml protinase K) and incubated in a water bath at 50 °C for 24 h with one change of ESP. The ESP was replaced with three volumes of THE (10 mM Tris-HCl, 10 mM EDTA, pH 8) containing 1 mM phenylmethylsulfonyl fluoride, then THE alone both at 50 °C for 90 minutes each. In the last step, plugs were transferred into 50 mM EDTA, pH 8 and keep at 4 °C until use.

Restriction Digestion, PFGE and Blotting: DNA inserts were cut into 24 µl plugs, each containing approximately 3-4 µg DNA. Each plug was pre-incubated twice in 250 µl of the appropriate restriction buffer, 30 minutes each on ice. The buffer was replaced with 100 µl buffer containing 50 units of the appropriate restriction enzyme, 2 mM spermidine and 0.1 mg/ml acetylated BSA. After 60 min pre-incubation on ice, the sample was incubated overnight at the required temperature. The digestion mix was replaced by 500 µl 50 mM EDTA, pH 8, to stop the reaction. The restriction enzymes used were: MluI, NotI, NruI, SacII, SalI and SfiI. The digested DNA was separated on 1% FastLane agarose contour-clamped homogeneous electrophoresis gel system

(Schwartz and Cantor, 1984) at 6.0 v/cm, with 60 to 110 sec. ramped switch time, for 22 h , at 14 °C, in 0.5 x TBE. After staining and photography, the gel was subjected to Southern blotting and probing (Concibido et al., 1994).

Results and Discussion

Examination of the crude extraction suspension, after staining with 2% aceto-carmine and microscopy and before embedding into low melting point agarose, showed the presence of many intact nuclei. The PFGE analysis also confirmed that most DNA migrated in the limiting mobility region of the gel, corresponding to the largest Saccharomyces cerevisiae chromosome. There was very little or no degradation of the DNA and most of the DNA was over two megabase in size. Restriction digestion with rare cutting methylation sensitive enzymes produced large fragments upon cleavage. The eight base pair recognition cutters (NotI and SfiI) produced larger fragments (generally greater than 400 kilobase pairs (Kb) than did six bp recognition cutters (MluI, NruI, SacII and SalI) (between 100 and 500 Kb).

Earlier techniques for the preparation of HMW DNA in plants based on protoplast isolation and lysis were not only difficult and costly, but also led to contaminating chloroplastic and mitochondrial DNA. This complicates PFGE analysis and map-based cloning studies (Martin et al., 1992). Here we describe HMW DNA preparation from nuclei in soybean, enabling us to conveniently manipulate DNA in the megabase size scale and perform parallel physical and genetic mapping analysis for a specific region of the soybean genome.

With this technique, nine DNA markers within 10 cM of the major SCN locus on linkage group G were analyzed. Detailed PFGE analysis was performed with a group of five DNA markers tightly linked to one another and the SCN resistance locus (Bng189, Bng122, Bng113, Bng126, and Bng30). Previously, these markers were found to be tightly linked in Phaseolus based on segregation analysis (Vallejos et al. 1992). In soybean, these markers span a genetic distance of approximately 6 cM, although Bng189 was not polymorphic and could not be mapped (Fig. 1). Nonetheless, Bng189 was shown to be physically linked to Bng122 in soybean at a distance of approximately 150 Kb or less by PFGE analysis. Bng126, Bng30 and Bng113 were also found to be physically clustered. The marker order was determined to be Bng126, Bng113 and Bng30 and the distance between them estimated at 100 and 200 Kb (Fig. 1). Comparing the results of physical and genetic mapping distance,

1 cM on the soybean genetic linkage map translates to 150 Kb in this region of the soybean genome.

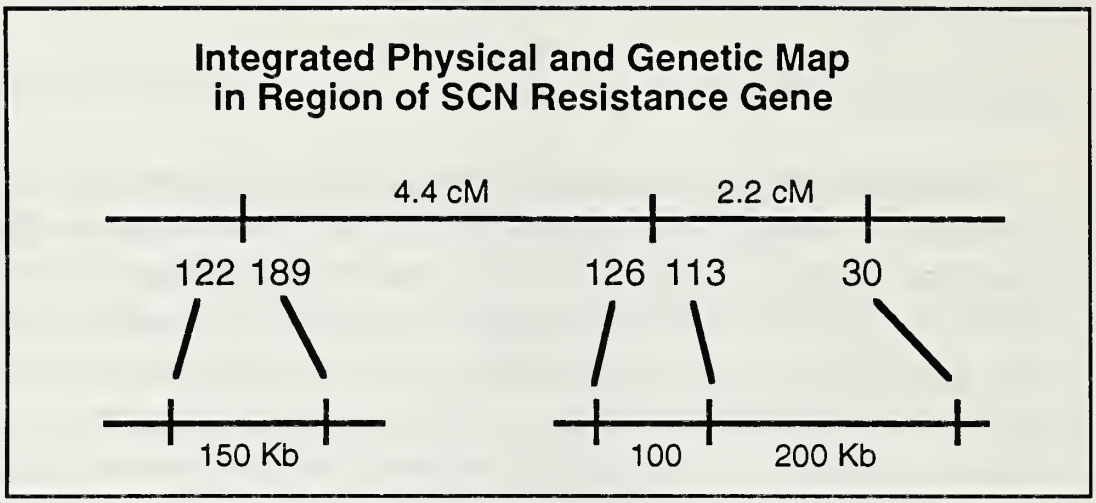

Figure 1

With these improved techniques for the genome analysis, it is now practical to physically bridge closely linked markers on the soybean genetic linkage map. Not only with this type of analysis provide a better understanding about the relationship between recombinational and physical distances, it will also establish an essential framework for the growing number of efforts at positional gene cloning in soybean.

References

Boutin S., N. Young, T. Olson, Z.H. Yu, R. Shoemaker, and C. Vallejos. 1995. Genome conservation among three legume genera detected with DNA markers. Genome, accepted for publication.

Concibido V., R.Denny, S. Boutin, R. Hautea, J. Orf, and N. Young. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode Heterodera glycines Ichinohe. Crop Sci. 34:240-246.

Diers, B.W., L. Mansur, J. Imsande, and R. Shoemaker. 1992. Mapping Phytophthora resistance loci in soybean with restriction fragment length polymorphism markers. Crop Science 32:377-383.

Keim P., B. Diers, T. Olson, and R. Shoemaker. 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735-742.

Martin G., M. Ganal, and S. Tanksley. 1992. Construction of yeast artificial chromosome library of tomato and identification of cloned segegating fragments linked to two disease resistance loci. Mol. Gen. Genet. 223:25-32.

Schwartz D. and C. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed-field gradient gel electrophoresis. Cell 37:67-75.

Vallejos C., N. Sakiyama, and C. Chase. 1992. A molecular marker-based linkage map of Phaseolus vulgaris L. Genetics 131:733-740.

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